

THE INDIAN JOURNAL OF MEDICAL
RESEARCH



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THE
INDIAN JOURNAL
OF
MEDICAL RESEARCH

PUBLISHED UNDER THE AUTHORITY OF
THE INDIAN RESEARCH FUND ASSOCIATION

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Vol. XIX, 1931-32.

PUBLISHED BY
THACKER, SPINK & CO., LTD., CALCUTTA
*Price per Volume Sixteen Rupees; Single Copies Five Rupees
(excluding postage)*

PRINTED BY
THACKER S PRESS & DIRECTORIES, LTD
6 Mangoe Lane, Calcutta

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RESEARCHES ON 'STONE'

Part IX.

STUDIES IN CALCIUM AND PHOSPHORUS METABOLISM

BY

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[Received for publication, December 20, 1930]

PREVIOUS work on urinary calculus in these laboratories (McCarrison, 1927 to 1930, Newcomb and Ranganathan, 1930, Ranganathan, 1930) have shown that the stones produced experimentally in albino rats were of two main varieties one, a well-defined, crystalline stone, consisting for the most part of ammonium magnesium phosphate, but containing no calcium, the other, an ill-defined, heterogeneous mass, containing an abundance of calcium, chiefly as carbonate, less often as hydroxide. These two types, though differing markedly from one another, had one feature in common complete absence of uric acid. The experimental diets, which gave rise to them, had deficiency of fat-soluble vitamins as their common factor. They fall into two broad categories those to which lime was added and those to which lime was not added. The former gave rise to stones which were invariably of the ammonium magnesium phosphate variety, the latter to stones which were invariably of the calcium carbonate or hydroxide variety. It seemed, therefore, that the addition of lime to the deficient diets profoundly affected calcium metabolism. Accordingly, an investigation was undertaken with the object of tracing the course of the ingested lime through the body. At the same time, the metabolism of phosphorus and magnesium, the two important elements occurring in the non-calcium stones, was also studied. To this end, various deficient

diets which had proved to be of high stone-producing potency were used (McCallison, 1927-1930)

Experimental.

The animals were kept in individual metabolism cages, specially designed in these laboratories for such metabolic studies. In the present studies great precautions were not taken to prevent fermentation of the urine and the faeces, as the investigation was limited to mineral metabolism alone.

The *basal diet* used in these experiments consisted of 97 grammes of white bread and 3 grammes of dried yeast to which were added 25 grains of slaked lime and 25 drops of an iodine solution containing one milligram of iodine per litre. Several modifications of this diet were made, and the effects of the change on the mineral metabolism studied. These modifications were as follows: additions of radiostoleum (vitamins A and D), sesame oil, milk, sodium phosphate, butter, sodium phosphate and vitamin A, vitamin A alone, sodium phosphate and radiostoleum, radiostoleum alone, or cod-liver oil to the basal diet. Two young albino rats in growing period were fed on each of these diets. The diets were made into a homogeneous, non-crumbling—neither too hard nor too soft—mass and pressed into small glass food-containers each holding 20 to 25 grammes. The animals were provided with distilled water *ad libitum*, when milk formed part of the diet, it was given in known quantity, the amount left unconsumed in the milk tube being measured the next morning.

The animals were fed at 11 A.M. daily and the following morning the food left unconsumed was picked out of the containers and weighed. The food-intake of each animal was thus arrived at, due allowance being made for loss of weight consequent on drying. This loss was slight, it ranged in the 13 diets used between 1.5 and 3 per cent.

The calcium, magnesium and phosphate contents of the various diets were estimated. The urines were collected daily into stoppered bottles and analyses made on representative samples of them collected during each week. In collecting the urines, urinary deposits were often noticed sticking to the stem of the funnel and to the bottom of the collecting flask. These deposits were dissolved in 5 to 10 c.c. of concentrated hydrochloric acid on the last day of each weekly collection, and added to the main bulk of the urines. An additional advantage of this procedure was that it held in solution salts which would otherwise have been thrown out of solution during the storage necessary before analyses could be completed by a single worker. Before the addition of the hydrochloric acid solution of the urinary deposits to the main bulk of the urines, aliquots of the representative samples were taken and utilized for chloride estimation. Total nitrogen, phosphate, calcium and magnesium were determined in the remainders. The faeces were collected daily at least two hours before the administration of food, care being taken to avoid them

contamination by food particles. They were weighed and then dried at 100°C for over 6 hours. When completely dry, they were again weighed, powdered and representative samples taken for analyses. Phosphates, calcium and magnesium were determined in them.

Methods of analysis

Food—A known weight of the food was ashed at low heat to constant weight, the ash taken up in hydrochloric acid, and calcium, magnesium and phosphates determined in the usual manner in the acid solution.

Urine—Chlorides were estimated by Volhard's method in representative samples of urine and the values computed as percentages of NaCl.

Nitrogen and phosphates were determined as follows. 1 c.c. of the urine was wet-ashed with 1.6 c.c. of concentrated, nitrogen-free sulphuric acid and a few crystals of potassium sulphate. To facilitate the digestion of the organic matter in the urine, it was found advisable to add about 0.2 to 0.3 c.c. of 'Merckozone' (12 or 20 volume strength). It was then mixed well, about ten minutes being allowed before digesting over a naked flame. These wet-ashings were made in special hard-glass Pyrex test tubes. After complete digestion, it was extracted with distilled water and the extracts made up to volume in a 50 c.c. measuring flask. An aliquot, brought approximately to the neutral point by addition of normal sodium hydroxide solution, was diluted to about 40 or 45 c.c. in a 50 c.c. measuring flask. Two c.c. of Nessler's reagent were then added, the volume made up and matched in a colorimeter against suitable standards treated likewise. Phosphates were determined in aliquots of the above by a modified Kuttner and Cohen's micro-method (Ranganathan, 1930). Values are expressed as percentages of P_2O_5 .

Calcium and magnesium were determined in the usual manner in the ash of the urines extracted with hydrochloric acid, and reported as percentages of CaO and MgO respectively.

Fæces—A weighed amount of the fæces was ashed in a porcelain crucible at not very high temperatures and the ash extracted with about 3 to 5 c.c. of concentrated hydrochloric acid. The acid extract and the subsequent washings with distilled water were passed through a filter paper and the filtrate made up to volume. It was invariably noticed that, while extracting the ash of the fæces with acid, a major portion of the ash remained undissolved, presumably containing the siliceous matter from the food. It was suspected that this insoluble portion of the ash contained a little of the elements sought for, viz., Ca, Mg and P and so, with a view to bring it into solution, it was subjected to an alkali-fusion. The fused mass was extracted first with water and finally with hydrochloric acid till distinctly acid. As in the soluble portion, Ca, Mg and P were estimated and the results expressed as percentages of CaO, MgO and P_2O_5 respectively.

It is thus possible from the above analysis to form a fairly accurate estimate of the actual intake of Ca, Mg and P, and of the respective amounts of them that are excreted through the urinary and intestinal tracts, and therefrom to arrive at the amounts retained in the body

Limitations of the experiment

Though much attention was bestowed to detail in an endeavour correctly to arrive at the balance of the three mineral ingredients, it is not to be understood that these experiments are devoid of the fallacies commonly associated with similar metabolic experiments. The precautions and experimental skill tend only to keep them at as low a minimum as possible. For instance, it is difficult to avoid the loss of urine voided during weighing of the animals. It is difficult also to collect all the particles of the faeces, especially in those instances where the animals pass semi-solid stools, portions of which inevitably stick to the wire-mesh. Washing them out and adding to the main bulk is not feasible, much less is it advisable.

Records of the intake of food, the volume of urine voided, the weight of the faeces excreted and the weekly body-weight were maintained for each animal.

Three series of experiments were undertaken, the observations being recorded in the three following parts —

Series I.

The mineral metabolism under this head was confined to the following four diets

Group I received a modified 'stock' diet,* consisting of 'atta' chapatties, sprouted gram, cabbage and milk,

Group II received the *basal* diet of white bread, yeast, lime and iodine (*vide infra*) without further addition,

Group III received the *basal* diet, wherein 2 parts of white bread were replaced by 2 of sesame oil containing half a drop of radiostoleum (300 Blue, B D H). The consumption of radiostoleum was approximately 0.06 of a drop per rat per day,

Group IV received the same diet as Group III but without the radiostoleum.

Weekly analyses of the urines and the faeces were made to find out the excretion of calcium, magnesium and phosphates by methods detailed in the preceding portion of the paper. These observations were extended to six weeks when the following alterations in the diets were made. The animals in

* The original 'stock' diet included in addition carrots and occasionally meat

Group I were given in addition slaked lime to the extent of 5 grains per rat per day (incorporated in 20 grammes of food) while the other three groups received milk in addition. This alteration in the diets was made with a view to finding out what changes, if any, were brought about in the excretion of lime by its addition to the 'stock' diet and what by making good the vitamin deficiencies of the other diets by the addition of milk. The observations of mineral metabolism, under the altered conditions, were carried out for a further six weeks. The results are set out in Tables I A, B and C and Table II. Table I gives the results as total excreted, while Table II gives the percentages in the samples of urines or faeces. The results for the faeces are reported on moisture-free samples.

TABLE I-A

Sample number		CALCIUM AS (CaO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Faeces mg	Total mg	Mg	Percentage of intake
Group I	1	828	11	113	124	704	85
	2	839	64	106	170	669	80
	3	829	33	113	146	683	83
	4	853	69	115	184	669	78
	5	867	56	131	187	680	79
	6	847	37	194	231	616	73
	7	2,137	50	761	811	1,326	62
	8	2,161	26	1,092	1,118	1,043	48
	9	1,989	52	858	910	1,079	54
	10	2,090	29	1,231	1,260	830	40
	11	2,260	50	1,552	1,602	658	29
	12	2,163	72	1,661	1,733	430	20
Mean for the first 6 weeks		844	45	129	174	670	80
Mean for the last 6 weeks		2,133	46.5	1,192.5	1,239	894	42.2

TABLE I-A—*contd*

Sample number		CALCIUM AS (CaO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
Group II	1	1,260	250	375	625	635	50
	2	1,010	131	269	400	610	60
	3	1,184	165	302	467	717	60
	4	945	186	229	415	530	56
	5	908	92	211	303	605	67
	6	208	10	60	70	138	67
	7	1,262	61	394	455	807	64
	8	1,363	138	575	713	650	48
	9	544	16	206	222	322	59
Group III	1	1,288	200	290	490	798	62
	2	1,296	194	278	472	824	64
	3	1,216	203	201	404	812	67
	4	1,310	377	336	713	597	46
	5	1,400	351	259	610	790	56
	6	1,376	597	275	872	504	37
	7	1,518	265	401	666	852	56
	8	1,604	51	537	588	1,016	63
	9	1,629	26	651	677	952	58
	10	1,618	33	710	743	875	54
	11	1,657	26	752	778	879	53
	12	1,670	56	1,088	1,114	526	32
Mean for the first 6 weeks		1,314	320	273	593.5	721	55.3
Mean for the last 6 weeks		1,616	75.5	690	766	850	52.7

TABLE I-A—concl'd

Sample number		CALCIUM AS (CaO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
Group IV	1	1,112	223	446	669	443	40
	2	1,068	156	349	505	563	53
	3	1,215	204	279	483	732	61
	4	1,150	196	248	444	706	61
	5	1,200	160	303	463	737	61
	6	1,170	88	321	409	761	65
	7	1,351	206	426	632	719	53
	8	1,396	57	495	552	844	61
	9	1,495	98	888	986	509	34
	10	1,375	45	734	779	596	43
	11	1,552	40	779	819	733	47
	12	1,486	69	729	798	688	46
Mean for the first 6 weeks		1,152.5	171	324	495.5	657	56.8
Mean for the last 6 weeks		1,442.5	86	675	761	681.5	47.2

It will be seen from Table I that there was very little excretion of calcium through the urinary tract of rats during the growing period of life when they were fed on the 'stock' diet. Even when the calcium content of the diet was greatly increased by the addition of slaked lime, the excretion of calcium in the urine was not appreciably affected, though the excretion through the bowels was greatly increased. These results are in conformity with those of Stewart and Percival (1928), and Telfer (1922) and Bauer, Albright and Aub (1930).

But the addition of lime to diets of which the chief deficiencies were fat-soluble vitamins and phosphates led to a great excretion of calcium through the urinary tract. The excretion of calcium through the bowels was also great. When the above deficiencies were partly corrected by the addition of milk, the urinary calcium decreased while the faecal calcium correspondingly increased.

TABLE I-B

PHOSPHATES (P ₂ O ₅)								
Sample number	Intake mg	OUTPUT			BALANCE OR RETENTION		CaO retained/ P ₂ O ₅ retained	
		Urine mg	Fæces mg	Total mg	Mg	Percentage of intake		
Group I	1	986	320	260	580	406	41	1 73
	2	980	448	180	628	352	36	1 90
	3	946	444	155	599	347	37	1 97
	4	1 103	484	212	696	407	37	1 64
	5	1,122	451	202	653	469	42	1 45
	6	1,039	497	340	837	202	20	3 05
	7	1,026	244	620	864	162	16	8 20
	8	1,037	152	858	1,010	27	3	38 70
	9	963	164	742	906	57	6	18 9
	10	1,006	157	1,004	1,161	—155	—15	
	11	1,081	118	1,024	1,142	—61	—6	
	12	1,038	138	1,106	1,244	—206	—20	
Mean for the first 6 weeks		1,029	441	225	666	364	35 5	1 96
Mean for the last 6 weeks		1,025	162	892	1,054 5	—29	—27	
Group II	1	392	7	182	189	203	52	3 13
	2	315	9	100	109	206	66	2 96
	3	370	12	71	83	287	78	2 50
	4	295	7	56	63	232	79	2 28
	5	283	12	83	95	188	67	3 22
	6	65	4	31	35	30	46	4 60
	7	494	34	240	274	220	45	3 66
	8	551	43	301	344	207	38	3 15
	9	219	10	123	133	86	39	3 75

It was suspected that the apparent discrepancies in the estimations of the urinary calcium of animals in Group IV, just before the addition of milk, were possibly due to the development of stone in one of the animals, which exhibited clinical symptoms of this malady—protrusion of the penis (which is a common symptom of stone) and loss of weight, this, it was thought, would lead to a storage of mineral salts. This suspicion was later confirmed, when at the post-mortem, conducted by the Pathological Department of these laboratories, stones were found both in the kidney and bladder, these weighed 19.2 and 95.0 mg respectively. Their compositions were as follows—

	Moist	N	P ₂ O ₅	CaO	MgO	CO ₂
Kidney	5.1	1.6	0.9	44.2	Trace	40.8
Bladder	3.6	1.3	1.4	43.0	1.5	36.5

The figures represent results on moisture-free samples

TABLE I-B—*contd*

Sample number		PHOSPHATES (P ₂ O ₅)						
		Intake mg	OUTPUT			BALANCE OR RETENTION		CaO retained/ P ₂ O ₅ retained
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake	
Group III	1	402	9	160	169	233	58	3.43
	2	404	7	76	83	321	80	2.56
	3	379	11	78	89	290	77	2.80
	4	412	7	68	75	337	82	1.77
	5	438	19	136	155	283	65	2.79
	6	429	23	136	159	270	63	1.87
	7	579	11	221	232	347	60	2.46
	8	609	15	265	280	329	54	3.10
	9	637	33	345	378	259	41	3.68
	10	634	59	344	403	231	36	3.78
	11	637	58	431	489	148	23	5.91
	12	639	95	458	553	86	13	6.10
Mean for the first 6 weeks		411	13	109	122	289	70.8	2.54
Mean for the last 6 weeks		622.5	45	344	389	233	37.8	4.17

TABLE I-B—concl'd

Sample number		PHOSPHATES (P_2O_5)						
		Intake mg	OUTPUT			BALANCE OR RETENTION		CaO retained/ P_2O_5 retained
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake	
Group IV	1	350	10	237	247	103	30	4 30
	2	334	8	73	81	253	76	2 23
	3	378	9	74	83	295	78	2·48
	4	358	7	57	64	294	82	2·40
	5	375	21	107	128	247	66	2·98
	6	365	17	152	169	196	54	3·89
	7	527	50	252	302	225	43	3 20
	8	572	60	309	369	203	35	4 15
	9	579	38	553	591	—12		
	10	545	59	372	431	114	21	5 21
	11	606	61	425	486	120	20	6 11
	12	580	70	348	418	162	28	4 24
Mean for the first 6 weeks		360	12	117	129	231	64 3	3·05
Mean for the last 6 weeks		568	56	376 5	433	135	24 5	

The amount of calcium excreted through the bowels in animals fed on the 'stock' diet exhibited a fairly close relation to the amount of phosphates in the fæces. In this diet the two ingredients, CaO and P_2O_5 , seemed to exist approximately in the ratio obtaining in calcium phosphate. Presumably, the calcium was excreted through the bowels mostly in the form of calcium phosphate. This relation of CaO to P_2O_5 was not so well observed in rats fed on the deficient diets, especially in the pre-milk period, showing thereby that a considerable part of the calcium was excreted in some form other than calcium phosphate, possibly as sulphate. Qualitative tests showed that the fæces of the deficiently-fed animals contained more sulphates than those of animals fed on the 'stock' diet.

TABLE I-C

Sample number		MAGNESIUM (MgO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
Group I	1	213	53	14	67	146	68.6
	2	218	8	10	18	200	91.9
	3	212	9	21	30	182	86.0
	4	234	6	32	38	196	83.9
	5	239	10	33	43	196	82.0
	6	221	25	170	195	26	11.8
	7	429	27	138	165	264	61.7
	8	434	75	312	387	47	10.8
	9	308	19	322	341	57	14.4
	10	419	70	267	337	82	19.6
	11	455	81	360	441	14	3.1
	12	434	72	385	457	-23	-5.3
Mean for the first 6 weeks		233	18.5	47	65	158	70.7
Mean for the last 6 weeks		428	57	297	355	73.5	18.2
Group II	1	239	85	6	91	148	62.0
	2	192	11	26	37	155	80.8
	3	225	28	18	46	179	79.7
	4	180	11	25	36	144	80.0
	5	172	17	25	42	130	75.7
	6	40	9	12	21	19	47.6
	7	247	50	50	100	147	59.7
	8	267	64	92	156	111	41.5
	9	107	4	47	51	56	52.5

TABLE I-C—contd

Sample number		MAGNESIUM (MgO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
Group III	1	245	67	14	81	164	67.0
	2	246	7	21	28	218	88.5
	3	231	19	19	38	193	83.7
	4	251	18	36	54	197	78.3
	5	266	32	49	81	185	69.7
	6	262	78	49	127	135	53.7
	7	296	51	66	117	179	60.4
	8	312	65	72	137	175	56.0
	9	319	16	110	126	193	60.7
	10	317	56	81	137	180	57.0
	11	324	48	116	164	160	49.5
	12	326	42	115	157	169	51.9
Mean for the first 6 weeks		250	37	31	68	182	73.5
Mean for the last 6 weeks		316	46	93	140	176	55.9

As regards phosphates there was, comparatively speaking, a far greater excretion of phosphates in the urine of rats fed on 'stock' diet than in that of others fed on the deficient diets. When lime was added to the 'stock' diet, the urinary phosphates rapidly decreased, while the faecal phosphates correspondingly increased. In the deficiently-fed rats, there was very little excretion of phosphates in the urine, but when milk was added, the urinary phosphates increased gradually. The faecal phosphates also increased. It is to be noted that in the post-milk period, there was a greater intake of phosphates.

A study of the excretions of CaO and P_2O_5 in rats fed on the four diets shows that the urinary excretion of phosphates seemed to be governed by the intake of CaO and P_2O_5 . If the diet be well-balanced as regards its calcium

TABLE I-C—*concd*

Sample number		MAGNESIUM (MgO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
Group IV	1	214	80	11	91	123	57.6
	2	203	13	19	32	171	84.0
	3	231	31	17	48	183	79.2
	4	218	20	30	50	168	77.0
	5	228	36	36	72	156	68.4
	6	222	39	41	80	142	64.0
	7	264	49	50	99	165	62.7
	8	275	42	74	116	159	54.1
	9	292	14	167	181	111	38.0
	10	270	48	90	138	132	48.8
	11	303	32	91	123	180	59.5
	12	292	26	64	90	202	69.2
Mean for the first 6 weeks		219	36.5	26	62	157	71.7
Mean for the last 6 weeks		283	35	89	124.5	158	55.4

and phosphates, there should theoretically be only very little excretion of calcium in the urine. A certain amount of the calcium taken in the food is retained in the system, the amount of phosphate that would go to balance the calcium retained would most probably be excreted through the kidneys, and possibly this accounts for the greater excretion of phosphates in the urine of rats fed on 'stock' diet. But in the deficiently-fed animals, where there was a preponderating excess of calcium over phosphates, very little of the phosphate was left, after combination with calcium in the bowels, to be excreted in the urine. Again, the greater urinary excretion of phosphates in 'stock' animals was due only to the relative amounts of CaO and P₂O₅ in the 'stock' diet and not to the content of fat-soluble vitamins in the diet, this was well

TABLE II

Sample number		OUTPUT OF CaO IN		OUTPUT OF P ₂ O ₅ IN		OUTPUT OF MgO IN	
		Urine	Fæces	Urine	Fæces	Urine	Fæces
		Percentage		Percentage		Percentage	
Group I	1	0 007	0·238	0·21	0 82	0 035	Trace
	2	0·032	0·53	0·23	1·02	0·004	Trace
	3	0 019	0 56	0 254	0·82	0·005	Trace
	4	0·034	0 53	0·238	1 00	0 003	0·09
	5	0·026	0·60	0·212	1 40	0·005	0·151
	6	0·018	1 04	0·245	2·04	0·012	1 03
	7	0 022	3 48	0 107	2·93	0·012	0·65
	8	0 014	4 30	0 079	3 45	0·039	1 25
	9	0·025	4 25	0·078	3·75	0 009	1 62
	10	0·015	4 75	0 081	3 94	0 036	1 05
	11	0·021	5 40	0·048	3 61	0·033	1·27
	12	0·032	6 48	0·060	4 36	0·032	1 52
Mean for the first 6 weeks		0·023	0 58	0·232	1 18	0·011	0·21
Mean for the last 6 weeks		0 022	4 44	0·076	3·67	0·027	1 23
Group II	1	0 18	2·82	0·005	1 88	0·062	Trace
	2	0·125	5 10	0 009	2 05	0 010	Trace
	3	0·140	5 80	0·011	1 42	0·024	Trace
	4	0·248	5 82	0 009	1 37	0·014	0·554
	5	0·131	6 12	0·018	2 51	0·024	0·752
	6	0·039	9 20	0 017	5 18	0·036	1 96
	7	0 041	7 48	0 023	4 71	0·034	0·98
	8	0 065	8·29	0·020	4 40	0·030	1 34
	9	0·039	6 30	0·025	3·78	0·009	1 44

TABLE II—contd

Sample number		OUTPUT OF CaO IN		OUTPUT OF P ₂ O ₅ IN		OUTPUT OF MgO IN	
		Urine	Fæces	Urine	Fæces	Urine	Fæces
		Percentage		Percentage		Percentage	
Group III	1	0.26	2.39	0.011	1.50	0.088	Trace
	2	0.303	5.43	0.011	1.57	0.011	Trace
	3	0.256	3.52	0.014	1.45	0.025	Trace
	4	0.305	5.32	0.005	1.12	0.015	0.46
	5	0.283	4.66	0.015	2.14	0.026	0.82
	6	0.339	4.40	0.013	2.38	0.044	0.86
	7	0.218	5.33	0.009	3.04	0.042	0.91
	8	0.032	7.89	0.009	3.95	0.041	1.06
	9	0.015	7.72	0.019	4.16	0.009	1.32
	10	0.020	8.04	0.036	3.95	0.034	0.93
	11	0.014	7.90	0.030	4.59	0.025	1.24
	12	0.028	10.56	0.049	4.50	0.021	1.12
Mean for the first 6 weeks		0.291	4.29	0.012	1.69	0.035	0.36
Mean for the last 6 weeks		0.055	7.91	0.025	4.03	0.029	1.10

shown by the behaviour of rats (Group III) receiving radiostoleum*. It was also shown by the rats on 'stock' diet in the post-lime period where a great imbalance between CaO and P₂O₅ was brought about by the addition of large amounts of calcium to the diet.

It will also be seen from Table I that, in rats on 'stock' diet, about 80 per cent of the calcium intake was retained in the system, while in the post-lime period, the retention fell to 42.2 per cent of the intake. However, the amount of calcium retained in the post-lime period was greater than in the

*It is possible, however, that the amount of fat-soluble vitamins added to the deficient diet in the form of radiostoleum may have been insufficient for the metabolic needs of the animals. But as will be seen later in series III, the same observations were noticed even when the radiostoleum content of the diet was increased sixteen-fold.

TABLE II—concl'd

Sample number		OUTPUT OF CaO IN		OUTPUT OF P O ₅ IN		OUTPUT OF MgO IN	
		Urine	Fæces	Urine	Fæces	Urine	Fæces
		Percentage		Percentage		Percentage	
Group IV	1	0.15	5.95	0.007	3.35	0.054	Trace
	2	0.134	6.45	0.007	1.41	0.011	Trace
	3	0.163	5.62	0.007	1.57	0.025	Trace
	4	0.141	5.60	0.005	1.32	0.015	0.55
	5	0.124	6.68	0.017	2.43	0.028	0.82
	6	0.071	6.91	0.014	3.38	0.031	0.91
	7	0.174	7.18	0.042	4.33	0.042	0.86
	8	0.043	7.96	0.060	5.02	0.031	1.20
	9	0.060	7.18	0.024	4.55	0.009	1.37
	10	0.030	8.65	0.039	4.43	0.032	1.06
	11	0.021	9.02	0.032	4.96	0.017	1.06
	12	0.037	9.50	0.038	4.58	0.014	0.84
Mean for the first 6 weeks		0.131	6.20	0.010	2.24	0.027	0.038
Mean for the last 6 weeks		0.061	8.25	0.039	4.65	0.024	1.07

pre-lime period, this increase being on the average from 670 to 894 milligrams of CaO. Again, in the pre-lime period, there was an average retention of 35.5 per cent of the intake of phosphates, while in the post-lime period, there was a negative balance amounting to 2.7 per cent of the intake. Further, the retention of MgO decreased with the addition of lime to the diet, from 70.7 per cent in the pre-lime period to 18.2 per cent of the intake in the post-lime period. Judging from the above results reported as percentages in Table II, it is seen that in rats on 'stock' diet, there was an average urinary excretion of 0.023 per cent of CaO during the pre-lime period. Even when the intake of CaO was greatly increased, the percentage of urinary CaO remained almost the same, the average percentage being 0.022. The addition of lime to the diet increased the percentage of faecal CaO, from 0.58 in the pre- to 4.44 in

the post-lime period. Again, the addition of lime decreased the percentage of urinary excretion of phosphates, this decrease being on the average from 0.232 to 0.076 per cent. There was a corresponding increase, from 1.18 to 3.67 per cent in the percentage of faecal phosphates. The percentage urinary excretion of MgO was increased from 0.011 to 0.027, while in the faeces, there was a more marked increase from 0.21 to 1.23 per cent.

The animals in Group II were of poor health for a major portion of the period they were under observation. They declined from the first week onwards till eventually they died before the completion of the sixth week (Fig IV). Others kept on the same diet for some time were substituted for them. They also were of declining health and hardly survived for three weeks, in spite of the addition of milk to their diet. The results are incorporated more for purposes of record than for instituting any comparison with the results obtained from other diets.

There was in the pre-milk period considerable excretion of CaO through the kidneys of animals in Group III, amounting on an average to 320 mg or about 25 per cent of the intake. When milk was added to the diet, the CaO excretion fell from 597 mg in the last week of the pre-milk period to 265 mgms in the first week of the post-milk period. In the eighth week, there was a further fall to 51 mg or to less than a fifth of the previous week's excretion. The faecal CaO increased markedly on the addition of milk, the increase being on the average from 273 to 690 mg. While a greater amount of CaO was retained in the system during the post-milk period, the retention, calculated as a percentage of the intake, showed a slight fall. The retention of P_2O_5 during the pre- and the post-milk periods, expressed as percentages of the intakes, showed a steady fall, from 70.8 to 37.8 per cent, while the amounts retained in the system did not exhibit this wide variation. The extra MgO added to the diet through the milk was mostly excreted in the faeces.

In Group IV, whose diet was the same as in Group III except that it contained no radiostoleum, the urinary excretion of CaO was appreciable, amounting on the average to 171 mg in the pre-milk period. The addition of milk reduced this excretion, the average being only 86 mg. The faecal CaO was increased from 324 mg in the pre- to 675 mg in the post-milk period. The amounts of CaO retained in the two periods were much the same, though the percentage retention showed a slight fall. The faecal phosphates increased markedly from 117 mg in the pre- to 377 mg in the post-milk period, so much so that the amount retained during the latter period was only half as much as that in the former. The extra amount of MgO added to the diet through the milk was mostly excreted in the faeces without materially altering the excretion in urine or the retention in the system.

More chlorides and less nitrogen were excreted in the urine by the deficiently-fed rats than by those fed on the 'stock' diet, the addition of

TABLE IV
Showing increase in body-weight, volume of urine voided and weight of faeces excreted

Group	Particulars	Sample number											
		1	2	3	4	5	6	7	8	9	10	11	12
I	Increase in body-weight, ^{gs}	41.0	26.0	39.0	35.0	22.0	20.0	18.0	24.0	27.0	19.0	9.0	18.0
	Urine voided, cc	153.0	195.0	175.0	204.0	213.0	203.0	228.0	192.0	210.0	195.0	246.0	230.0
	Fæces excreted (moist), ^{gs}	31.7	25.5	27.9	32.3	37.9	25.8	30.7	38.7	30.5	38.7	45.7	42.8
	Fæces excreted (dry), ^{gs}	19.4	17.7	18.9	21.2	21.5	16.7	21.2	24.9	19.8	25.5	28.4	25.4
	Increase in body-weight, ^{gs}	8.0	2.0	—12.0	—4.0	—5.0		28.0	9.0				
II	Urine voided, cc	137.0	105.0	118.0	75.0	70.0	25.0	148.0	212.0	42.0		.	
	Fæces excreted (moist), ^{gs}	9.7	7.0	7.0	5.9	4.9	0.9	6.9	8.8	4.7			
	Fæces excreted (dry), ^{gs}	5.8	4.9	5.0	3.9	3.3	0.6	5.1	6.9	3.3			
	Increase in body-weight, ^{gs}	15.0	17.0	13.0	30.0	20.0	16.0	17.0	13.0	18.0	15.0	9.0	15.0
	Urine voided, cc	76.0	64.0	79.0	124.0	124.0	176.0	122.0	158.0	172.0	163.0	194.0	196.0
III	Fæces excreted (moist), ^{gs}	10.7	7.8	10.4	11.5	10.5	10.5	11.2	10.2	10.4	11.4	14.1	15.7
	Fæces excreted (dry), ^{gs}	5.9	4.8	5.4	6.1	5.3	5.7	7.3	6.7	8.3	8.7	9.4	10.2
	Increase in body-weight, ^{gs}	18.0	12.0	13.0	13.0	—2.0	—8.0	0.0	6.0	24.0	—7.0	9.0	4.0
	Urine voided, cc	149.0	116.0	125.0	139.0	128.0	124.0	118.0	134.0	162.0	150.0	190.0	185.0
	Fæces excreted (moist), ^{gs}	7.1	7.8	7.0	6.9	6.9	6.1	7.2	8.3	15.2	9.9	10.6	9.7
IV	Fæces excreted (dry), ^{gs}	5.0	5.2	4.7	4.3	4.4	4.5	5.8	6.2	12.2	8.4	8.6	7.6

milk did not cause any appreciable difference. More urine and faeces were excreted on 'stock' diet than on the deficient diets, the same result was observed even after the dietetic deficiency was partly corrected by the addition of milk.

The urinary and faecal excretions of CaO and P_2O_5 in Groups I, III and IV are charted out in Charts 1, 2 and 3. The body-weights of the animals are given in Chart 4. Tables III, IV and V give food consumption of the animals in the different groups, weekly increase in body-weights, urine voided and faeces excreted, and the total excretion of chlorides and nitrogen in the urines.

TABLE V
Showing urinary excretion of chlorides and nitrogen

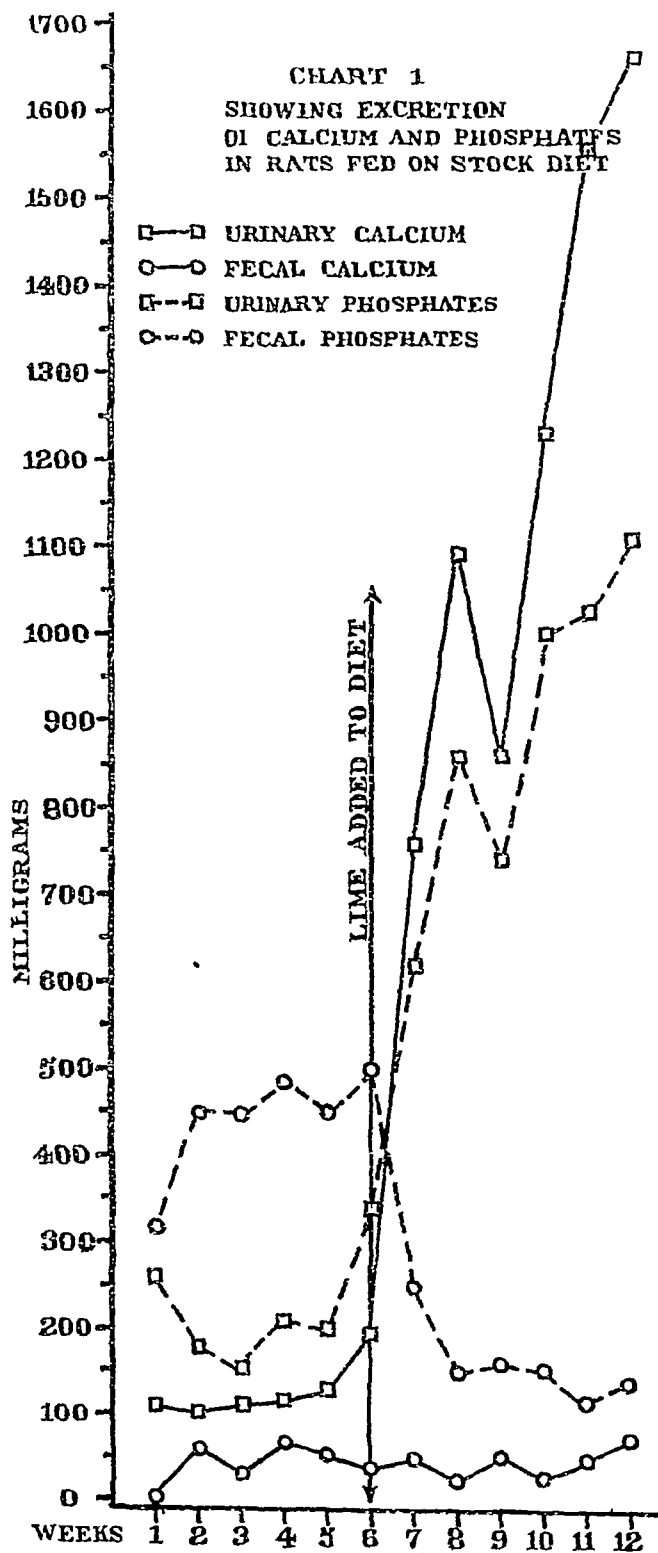
Sample number	GROUP I		GROUP II		GROUP III		GROUP IV	
	Chlorides gs	Nitrogen gs	Chlorides gs	Nitrogen gs	Chlorides gs	Nitrogen gs	Chlorides gs	Nitrogen gs
1	0.29	1.80	1.48	0.95	1.38	0.85	1.31	1.00
2	Trace	1.68	1.16	0.91	1.12	0.65	1.05	0.81
3	0.23	1.78	1.45	1.00	1.60	0.88	1.45	0.90
4	0.26	2.35	0.88	0.72	1.69	1.12	1.54	0.97
5	0.69	2.37	0.82	0.83	1.86	1.14	1.42	0.98
6	0.65	2.55	0.29	0.18	2.64	0.82	1.38	0.83
7	0.29	2.88	2.21	1.26	1.42	0.98	1.15	1.07
8	0.25	2.41	3.04	1.24	1.90	1.26	1.05	1.11
9	0.50	2.29	0.55	0.32	2.01	1.44	1.68	1.14
10	0.39	2.16			2.12	1.45	1.37	1.17
11	0.32	2.52			2.02	1.84	1.50	1.43
12	0.44	2.92			1.90	1.86	1.14	1.31

Series II

The mineral metabolism under this head was confined to the following four diets —

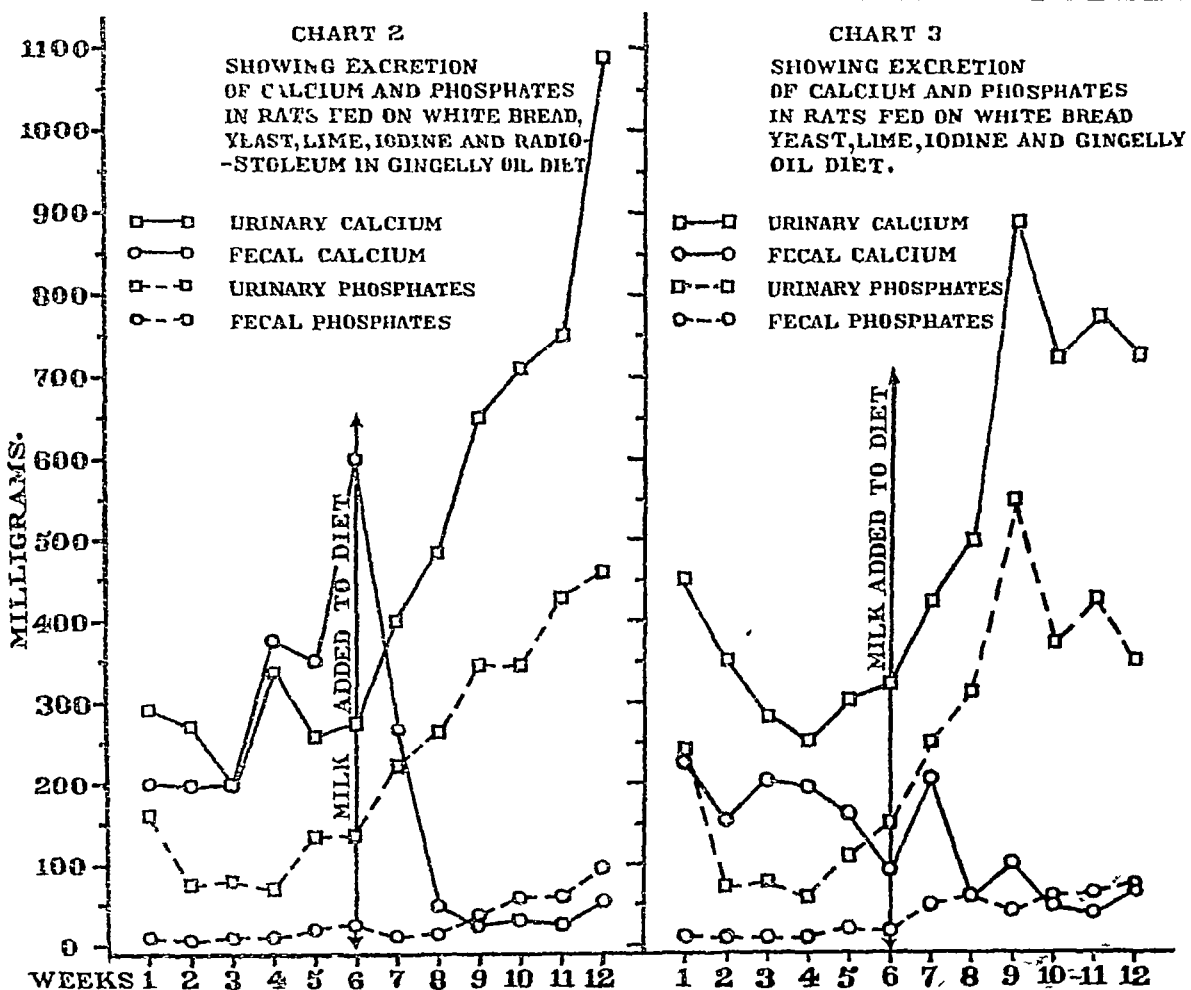
Group I received the *basal* diet of white bread, yeast, lime and iodine without further additions,

Group II received the *basal* diet plus milk *ad libitum*,



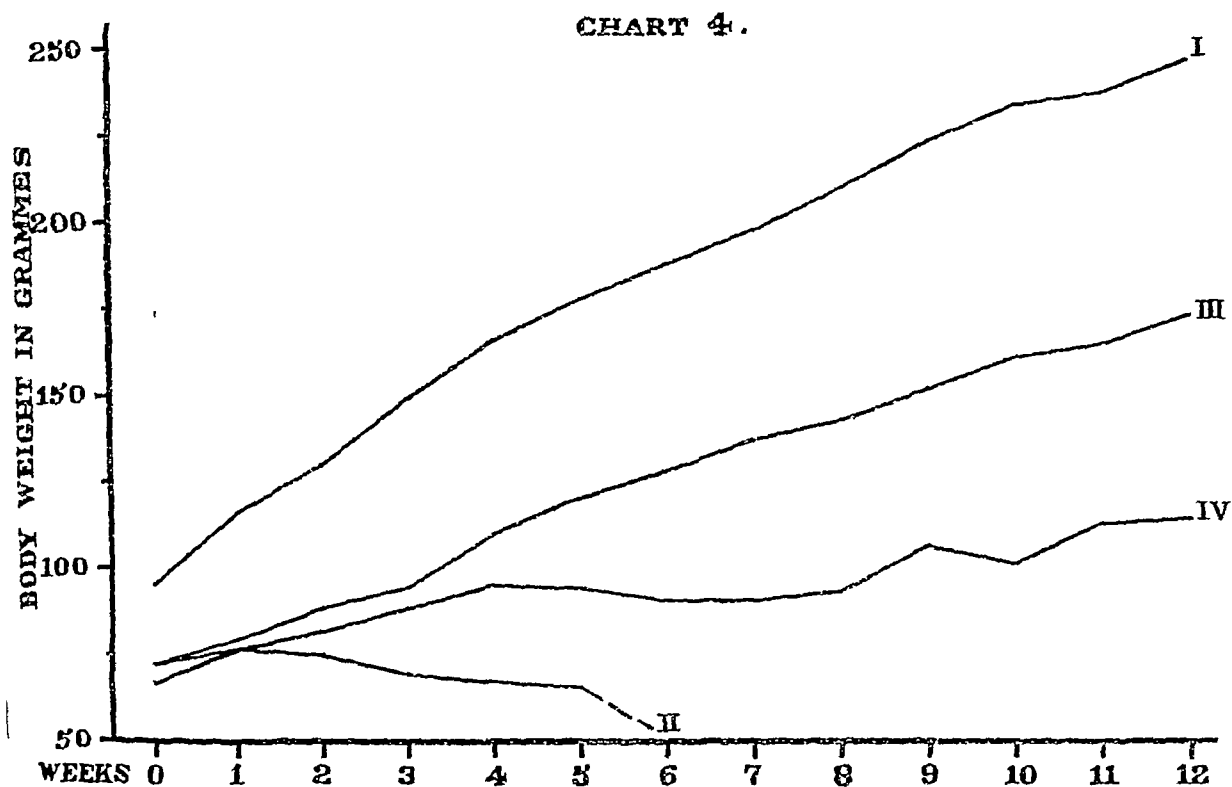
Group III received the *basal diet plus* sodium phosphate in amounts approximately equivalent to the amount of calcium in the diet so as to facilitate the formation of calcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$,

Group IV received the *basal diet plus* butter to the extent of 20 per cent of the food



It was suspected, as a result of the metabolic studies recorded under the first series of experiments, that if the diet rich in calcium were also to contain an abundance of phosphates, in quantities sufficient to form calcium phosphate, then very little of the calcium ingested would pass through the kidneys, while

most of it would pass through the bowels. Further, it had been noticed in the first series that addition of milk to calcium-rich diets was able to divert the course of a major part of the calcium from the urine to the faeces. In order to determine whether this was achieved solely by the fat-soluble vitamins



of the milk, or by its phosphate content alone, or by the conjoint effect of both, as suggested by Colonel McCarrison, the above four diets were employed. The conjoint effect can be observed in Group II, while the individual effects of either phosphates or fat-soluble vitamins can be observed in Groups III and IV respectively.

As in the first series weekly analyses of the urines and the faeces were made to find out the excretions of CaO , MgO and P_2O_5 . These observations were extended to nine weeks. The diets were also analysed for their CaO , MgO and P_2O_5 contents to evaluate the intake of the three mineral salts. The results, as total excreted, are reported in Table VI A, B and C, while Table VII gives the same as percentages of the urines or the faeces. The results for the faeces are reported as before on moisture-free samples.

TABLE VI-A

Sample number		CALCIUM AS (CaO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
Group I	1	930	10	318	328	602	64.9
	2	1,020	13	341	354	666	65.1
	3	920	118	230	348	572	62.2
	4	1,188	152	282	434	754	63.5
	5	1,270	129	291	420	850	67.0
	6	1,238	166	222	388	850	68.9
	7	1,160	176	195	371	789	68.0
	8	1,252	56	249	305	947	75.8
	9	1,330	41	316	357	973	73.1
Mean		1,145	96	272	367	778	66.3
Group II	1	1,460	45	243	288	1,172	80.3
	2	1,781	112	387	499	1,282	72.0
	3	1,782	96	421	517	1,265	71.1
	4	1,901	260	589	849	1,052	55.3
	5	1,947	247	772	1,019	928	47.5
	6	2,014	95	813	908	1,106	51.8
	7	1,985	130	722	852	1,133	56.9
	8	2,214	58	1,034	1,092	1,122	50.7
	9	2,281	52	1,047	1,099	1,182	51.8
Mean		1,929	122	670	791	1,138	59.7
Group III	1	1,306	33	509	542	764	58.7
	2	1,660	93	915	1,008	652	39.4
	3	1,800	87	895	982	818	45.4
	4	1,922	66	1,189	1,255	667	34.7
	5	1,980	81	1,128	1,209	771	39.0
	6	1,998	110	1,095	1,205	793	39.8
	7	1,984	142	990	1,132	852	43.0
	8	1,962	116	839	955	1,007	51.2
	9	1,982	79	1,004	1,083	899	45.3
Mean		1,844	90	952	1,041	803	44.1
Group IV	1	1,038	58	304	362	676	65.0
	2	1,020	28	472	500	520	51.0
	3	928	54	237	291	637	68.8
	4	950	69	251	320	630	66.3
	5	1,000	54	207	261	739	73.9
	6	1,064	67	198	265	799	74.8
	7	1,084	112	176	288	796	73.4
	8	1,136	42	273	315	821	72.6
	9	1,156	29	287	316	840	72.8
Mean		1,042	57	267	324	718	68.7

TABLE VI-B

Sample number		PHOSPHATES (P ₂ O ₅)						
		Intake mg	OUTPUT			BALANCE OR RETENTION		C/O retention/ P ₂ O ₅ reten- tion
			Urine mg	Faeces mg	Total mg	Mg	Percentage of intake	
Group I	1	272	2	110	112	160	58.9	3.77
	2	298	3	88	91	207	69.6	2.23
	3	269	7	80	87	182	67.8	3.15
	4	346	11	85	96	250	72.2	3.02
	5	371	15	88	103	268	72.2	3.17
	6	361	20	74	94	267	73.9	3.18
	7	338	22	72	94	244	72.0	3.23
	8	365	16	72	88	277	75.9	3.41
	9	389	13	88	101	288	74.1	3.38
Mean		334	12	84	96	238	70.7	3.28
Group II	1	575	10	99	109	466	81.1	2.51
	2	675	24	79	103	572	84.9	2.24
	3	676	22	171	193	483	71.6	2.62
	4	715	29	208	237	478	66.9	2.20
	5	740	31	262	293	447	60.4	2.07
	6	764	36	286	322	442	57.9	2.50
	7	749	25	253	278	471	63.0	2.40
	8	822	34	326	360	462	56.1	2.43
	9	842	39	300	339	503	59.9	2.35
Mean		729	28	220	248	480	66.8	2.37
Group III	1	930	5	222	227	703	75.8	1.09
	2	1,180	22	268	290	890	75.5	0.74
	3	1,282	55	261	316	966	75.2	0.85
	4	1,370	100	296	396	974	71.1	0.69
	5	1,420	98	243	341	1,079	76.0	0.72
	6	1,422	131	486	617	805	56.7	0.99
	7	1,418	158	494	652	766	54.0	1.12
	8	1,400	122	369	491	909	64.9	1.11
	9	1,418	84	418	502	916	64.7	0.98
Mean		1,316	86	340	426	890	68.2	0.92
Group IV	1	304	5	82	87	217	71.4	3.12
	2	299	7	151	158	141	47.3	3.69
	3	271	9	56	65	206	76.0	3.09
	4	278	13	53	66	212	76.4	2.98
	5	293	16	50	66	227	77.7	3.25
	6	311	15	49	64	247	79.7	3.23
	7	318	19	57	76	242	76.2	3.28
	8	332	21	108	129	203	61.2	4.05
	9	338	11	100	111	227	67.2	3.70
Mean		305	13	78	91	214	70.3	3.38

TABLE VI-C

Sample number		MAGNESIA (MgO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
Group I	1	163	5	63	68	95	58.2
	2	179	9	55	54	115	64.3
	3	161	31	38	69	92	57.0
	4	208	43	44	87	121	58.2
	5	223	68	46	114	109	48.9
	6	217	45	39	84	133	61.3
	7	203	24	32	56	147	72.2
	8	219	23	28	51	168	77.0
	9	233	21	34	55	178	76.4
Mean		201	30	42	72	129	63.5
Group II	1	271	28	50	78	193	71.1
	2	328	76	86	172	156	47.5
	3	330	57	80	137	193	58.5
	4	350	107	88	195	155	44.3
	5	359	113	117	230	129	36.0
	6	371	108	113	221	150	40.4
	7	365	58	89	147	218	59.8
	8	406	59	120	179	227	56.0
	9	418	65	101	166	252	60.3
Mean		355	75	95	169	186	52.7
Group III	1	229	20	108	128	101	44.2
	2	291	45	165	210	81	27.9
	3	316	26	134	160	156	49.4
	4	337	15	152	167	170	50.6
	5	347	15	153	168	179	51.6
	6	350	13	135	148	202	57.9
	7	349	15	110	125	224	64.0
	8	345	18	89	107	238	69.0
	9	348	13	107	120	228	65.6
Mean		324	20	128	148	175	53.4
Group IV	1	182	21	97	118	64	35.3
	2	178	20	86	106	72	40.5
	3	162	26	50	76	86	53.0
	4	166	18	47	65	101	60.8
	5	176	22	44	66	110	62.7
	6	186	17	33	50	136	73.0
	7	190	24	54	78	112	59.0
	8	198	29	52	81	117	59.0
	9	202	24	53	77	125	61.8
Mean		182	22	57	80	103	56.1

TABLE VII

Sample number		OUTPUT OF CaO IN		OUTPUT OF P ₂ O ₅ IN		OUTPUT OF MgO IN	
		Urine	Fæces	Urine	Fæces	Urine	Fæces
		Percentage		Percentage		Percentage	
Group I	1	0.101	7.37	0.018	2.64	0.051	1.53
	2	0.089	10.80	0.018	2.83	0.061	1.62
	3	0.318	8.00	0.018	2.83	0.084	1.34
	4	0.287	8.71	0.020	2.68	0.082	1.40
	5	0.172	9.57	0.020	2.95	0.091	1.55
	6	0.154	7.81	0.019	2.65	0.042	1.42
	7	0.154	6.81	0.019	2.60	0.021	1.14
	8	0.052	8.20	0.016	2.43	0.022	0.95
	9	0.047	9.30	0.014	2.64	0.024	1.01
Mean		0.153	8.51	0.018	2.69	0.053	1.33
Group II	1	0.076	5.18	0.016	2.20	0.046	1.11
	2	0.077	5.39	0.017	1.13	0.052	1.26
	3	0.058	5.88	0.014	2.43	0.034	1.13
	4	0.135	7.58	0.015	2.70	0.055	1.15
	5	0.142	8.20	0.018	2.84	0.065	1.26
	6	0.049	8.40	0.018	3.00	0.056	1.18
	7	0.078	7.87	0.015	2.80	0.034	0.99
	8	0.034	8.70	0.019	2.79	0.033	1.02
	9	0.032	9.62	0.025	2.81	0.041	0.95
Mean		0.076	7.42	0.017	2.52	0.046	1.12
Group III	1	0.109	7.58	0.017	3.41	0.066	1.66
	2	0.105	11.84	0.024	3.56	0.051	1.99
	3	0.059	12.00	0.038	3.54	0.018	1.82
	4	0.030	14.20	0.046	3.60	0.007	1.84
	5	0.043	15.40	0.052	3.35	0.008	2.11
	6	0.052	14.24	0.062	6.40	0.013	1.78
	7	0.071	12.44	0.079	6.30	0.007	1.40
	8	0.068	13.60	0.072	6.04	0.011	1.43
	9	0.064	13.40	0.068	5.62	0.010	1.44
Mean		0.067	12.74	0.051	4.65	0.021	1.72
Group IV	1	0.225	4.02	0.018	1.12	0.079	1.31
	2	0.054	7.80	0.014	2.54	0.039	1.35
	3	0.080	4.15	0.013	1.01	0.039	0.90
	4	0.087	4.65	0.017	1.01	0.023	0.89
	5	0.060	4.93	0.017	1.24	0.024	1.08
	6	0.086	4.55	0.020	1.15	0.022	0.77
	7	0.122	3.95	0.020	1.32	0.026	1.26
	8	0.036	5.84	0.018	2.37	0.025	1.14
	9	0.039	7.38	0.015	2.62	0.032	1.38
Mean		0.088	5.25	0.017	1.60	0.034	1.12

It will be seen from Table V that in rats fed on the *basal* diet without further additions (Group I), about a fifth of the intake of CaO was excreted in the urine, a fourth in the faeces and the rest retained in the system. Most of the phosphates of the diet was retained in the system, the retention being on the average about 71 per cent of the intake, of the 29 per cent excreted, most of it was through the bowels while only a small fraction was excreted through the kidneys. About 16 per cent of the intake of MgO was excreted in the urine, about 20 per cent in the faeces and the balance of about 64 per cent retained in the system.

When milk was added to the *basal* diet, the urinary excretion of CaO averaged 122 mg or only about 6 per cent of the intake. The same result, expressed as percentage of the urine, was found to be 0.076 as compared with 0.153 per cent in the urine of Group I. About two-thirds of the intake of phosphates was retained in the system, while about 4 per cent was excreted in the urine and the balance in the faeces.

When instead of milk, sodium phosphate in amounts approximately equivalent to the calcium content of the diet [to form $\text{Ca}_3(\text{PO}_4)_2$] was added to the *basal* diet, the excretion of CaO in the urine was only 4.9 per cent of the intake or 0.067 when expressed as percentage of the urine. About 52 per cent of the intake of CaO was excreted in the faeces, while the remaining 44 per cent was retained in the system. There was a greater excretion of urinary phosphates in this group as compared with the previous two groups, the excretion being, on the average, about 86 mg. About 68 per cent of the intake of phosphates was retained in the system.

When butter was added to the *basal* diet, the urinary excretion of CaO was, on the average, 57 mg or a little over 5 per cent of the intake. But there was as high a retention of CaO, viz., 68.7 per cent of the intake, as in the *basal* diet itself. The urinary excretion of phosphates was very little, being about 4 per cent of the intake, while about 70 per cent of the intake was retained in the system.

Slight changes were effected in the amount of butter added to the diet. Up to the end of the sixth week, the animals were receiving butter at the rate of 20 per cent of their diet or about 4 grammes per rat per day (incorporated in 20 grammes of food). In the middle of the seventh week, the percentage was reduced by one-half, viz., to 10 per cent, during the eighth week to 5 per cent, and during the last week butter was totally cut out from the diet. It was seen that the urinary excretion of CaO which was as high as 112 mg in the seventh week, rapidly fell to 42 mg in the eighth week, and to 29 mg in the ninth week. Synchronous with this decrease in the urinary output of CaO, the animals grew better during the last three weeks, they had better appetites and, consequently, there was a greater intake of CaO through the diet.

The retention of MgO was in all the four groups much the same, ranging from 53 to 63 per cent of the intake.

Post-mortem findings—At the end of the experiment the animals were killed and subjected to a complete post-mortem examination in the Pathological Department of these laboratories. Vesical calculus was found in one of the animals on the *basal* diet, the weight of the dry stones was 31.3 mg. On analysis, they were found to contain Moisture 11.5, Total nitrogen 2.2, P_2O_5 1.36, CaO 34.2, MgO nil and CO_2 Trace, the figures represent results in percentages on moisture-free stone.

TABLE VIII
Showing food consumption

Sample number	GROUP I	GROUP II		GROUP III	GROUP IV
	Food, gs	Food, gs	Milk, c c	Food, gs	Food, gs
1	130.7	150.6	198.1	183.5	174.4
2	143.2	193.1	206.7	232.9	171.8
3	129.1	194.0	206.5	253.0	156.0
4	166.7	208.6	212.2	269.8	159.9
5	177.9	210.7	227.4	278.4	168.5
6	173.5	219.7	231.8	279.8	178.9
7	162.4	217.2	226.3	279.9	182.2
8	175.7	246.8	234.3	275.3	190.5
9	186.2	257.3	230.6	279.2	194.2

TABLE IX
Showing increase in body-weight, volume of urine voided and weight of faeces excreted

Particulars	Sample number								
	1	2	3	4	5	6	7	8	9
<i>Group I</i>									
Increase in body-weight, gs	10.0	2.0	4.0	6.0	7.0	8.0	4.0	8.0	5.0
Urine voided, c c	10.0	15.0	37.0	53.0	75.0	108.0	114.0	106.0	88.0
Faeces excreted (moist), gs	5.6	4.2	3.8	4.6	4.0	3.6	4.1	4.3	4.1
Faeces excreted (dry), gs	4.15	3.10	2.82	3.18	2.98	2.77	2.78	2.96	3.33

TABLE IX—concl'd

Particulars	Sample number								
	1	2	3	4	5	6	7	8	9
<i>Group II</i>									
Increase in body-weight, gs	120	39 0	320	31 0	32 0	22 0	20 0	25 0	150
Urine voided, cc	600	146 0	166 0	193 0	174 0	194 0	167 0	176 0	161 0
Fæces excreted (moist), gs	70	101	9 0	10 9	14 2	121	122	15 6	147
Fæces excreted (dry), gs	4 50	7 00	7 05	7 68	9 23	9 56	9 05	11 65	10 07
<i>Group III</i>									
Increase in body-weight, gs	12 0	29 0	29 0	190	320	11 0	20 0	14 0	30
Urine voided, cc	300	89 0	146 0	218 0	188 0	214 0	2010	170 0	124 0
Fæces excreted (moist), gs	97	125	9 8	13 1	10 9	11 1	11 5	8 5	102
Fæces excreted (dry), gs	6 50	7 55	7 36	8 25	7 24	7 60	7 86	6 10	7 43
<i>Group IV</i>									
Increase in body-weight, gs		1 10	9 0	3 0	5 0	8 0	2 0	7 0	00
Urine voided, cc		26 52	68 0	79 0	91 0	78 0	920	118 0	74 0
Fæces excreted (dry), gs	8 8	7 5	6 5	6 3	5 0	5 7	5 8	5 7	4 9
Fæces excreted (moist), gs	7 35	5 95	5 57	5 26	4 07	4 23	4 28	4 57	3 83

TABLE X
Showing urinary excretion of chlorides and nitrogen

Sample number	GROUP I		GROUP II		GROUP III		GROUP IV	
	Chlorides gs	N gs	Chlorides gs	N gs	Chlorides gs	N gs	Chlorides gs	N gs
1	0 11	0 06	0 39	0 30	0 55	0 32	0 37	0 26
2	0 24	0 20	1 04	0 78	1 56	0 77	0 51	0 27
3	0 65	0 29	0 85	0 73	1 71	0 78	0 46	0 24
4	0 60	0 48	1 37	0 91	2 27	1 06	0 46	0 31
5	0 64	0 53	1 48	1 00	2 20	1 00	0 45	0 37
6	0 85	0 51	1 64	1 08	2 83	0 77	0 56	0 34
7	0 81	0 48	1 74	1 07	2 41	0 98	0 78	0 37
8	0 62	0 42	1 60	0 91	1 77	0 66	0 84	0 40
9	0 63	0 47	1 26	1 03	1 45	0 79	0 68	0 41

Series III.

The mineral metabolism in this series was restricted to the following six diets —

Group I received the *basal* diet *plus* sodium phosphate in amounts equivalent to the calcium content of the diet to form $\text{Ca}_3(\text{PO}_4)_2$,

Group II received the *basal* diet *plus* sodium phosphate as in Group I *plus* vitamin A concentrate [vitamin A oil, special (150 Blue), without added vitamin 'D,' British Drug Houses, Ltd] in the proportion of one drop per rat per day,

Group III received the *basal* diet *plus* vitamin A concentrate as in Group II,

Group IV received the *basal* diet *plus* sodium phosphate as in Group I *plus* radiostoleum (300 Blue, B D H) in the proportion of one drop per rat per day,

Group V received the *basal* diet *plus* radiostoleum as in Group IV,

Group VI received the *basal* diet *plus* sodium phosphate as in Group I *plus* cod-liver to the extent of 2 per cent of the diet

A relatively high urinary excretion of calcium and a low urinary excretion of phosphates having been observed in Group III (receiving the basal diet *plus* radiostoleum) of the first series of experiments, it was thought that this might have been due to the insufficient provision of fat-soluble vitamins. Hence in the present series, the amount of vitamin preparations, either as vitamin A concentrate or as radiostoleum, was increased nearly sixteen-fold. With a view to finding out the influence, if any, of vitamin D on this mineral metabolic study, diets containing vitamin A alone, and others containing both vitamins A and D were used. A diet with cod-liver oil (Group VI) was included to evaluate the efficiency of the proprietary vitamin preparations of the B D H in terms of cod-liver oil.

It may be mentioned here that the appetite of the animals receiving sodium phosphate in their diets, viz, those in Groups I, II, IV and VI was so great that the 20 grammes of food usually given was not sufficient. Therefore, the amount of food given was increased to 25 grammes per rat, but still containing only the 5 grains of lime and 5 drops of the iodine solution.

As before, weekly analyses of the urines and the faeces were made to find out the respective excretions of CaO , MgO and P_2O_5 . These observations were extended to six weeks. The diets, too, were analysed for their CaO , MgO and P_2O_5 contents. The results obtained, expressed in milligrams as total excreted, are set out in Table XI A, B and C and the same, calculated as percentages of either the urines or the faeces, are set out in Table XII. The results for the faeces are reported on moisture-free samples.

It is seen from Tables XI and XII that on a *basal* diet *plus* sodium phosphate there was an excretion of about 6.1 per cent of CaO through the kidneys and about 6.1 per cent through the bowels, leaving 32.6 per cent of the intake to be retained in the system. About 5.9 per cent of the intake

TABLE XI-A

Group and diet	Sample number	CALCIUM (AS CaO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
I Basal diet plus sodium phosphate	1	2,150	71	1,288	1,359	791	36.8
	2	2,295	100	1,376	1,476	819	35.6
	3	2,300	99	1,458	1,557	743	32.3
	4	2,340	182	1,480	1,662	678	28.9
	5	2,360	179	1,550	1,729	631	26.8
	6	2,355	213	1,320	1,533	822	34.9
	Mean	2,300	141	1,412	1,553	747	32.6
II Basal diet plus sodium phosphate plus vitamin A concentrate	1	2,265	64	1,430	1,494	771	34.0
	2	2,420	120	1,500	1,620	800	33.1
	3	2,455	98	1,434	1,532	923	37.5
	4	2,510	244	1,314	1,558	952	38.0
	5	2,500	263	1,538	1,801	699	27.9
	6	2,535	165	1,528	1,693	842	33.4
	Mean	2,448	159	1,457	1,616	831	34.0
III Basal diet plus vitamin A concentrate	1	1,022	67	470	537	485	47.5
	2	1,080	92	455	547	533	49.4
	3	1,014	253	619	872	142	14.0
	4	1,146	392	673	1,065	81	7.1
	5	1,010	354	597	951	59	5.9
	6	1,174	230	373	603	571	49.0
	Mean	1,074	231	531	763	312	28.8
IV Basal diet plus sodium phosphate plus radiostoleum	1	2,230	63	1,238	1,301	929	41.6
	2	2,290	73	1,244	1,317	973	42.7
	3	2,220	105	1,390	1,495	725	32.7
	4	2,325	233	1,520	1,753	572	24.6
	5	2,355	140	1,258	1,398	957	40.5
	6	2,410	305	1,460	1,765	645	26.8
	Mean	2,305	153	1,352	1,505	800	34.8

TABLE XI-A—concl'd

Group and diet	Sample number	CALCIUM AS (CaO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage mg
V Basal diet plus radiostoleum	1	1,220	138	387	525	695	57.0
	2	1,160	22	335	357	803	69.3
	3	1,142	141	503	644	498	43.5
	4	1,140	221	531	752	388	34.0
	5	1,054	156	557	713	341	32.5
	6	1,098	142	445	587	511	46.7
	Mean	1,136	137	460	596	539	47.2
VI Basal diet plus sodium phosphate plus cod-liver oil	1	2,228	30	1,318	1,348	880	39.5
	2	2,430	18	1,352	1,370	1,060	43.5
	3	2,400	50	1,440	1,490	910	38.0
	4	2,400	86	1,546	1,632	768	31.9
	5	2,470	30	1,540	1,570	900	36.5
	6	2,420	89	1,364	1,453	967	39.9
	Mean	2,391	51	1,427	1,477	914	38.2

TABLE XI-B

Sample number		PHOSPHATES (P.O ₅)					CaO retained/ P ₂ O ₅ retained	
		Intake mg	OUTPUT			BALANCE OR RETENTION		
			Urine mg	Fæces mg	Total mg	Mg		Percentage of intake
Group I	1	1,500	73	540	613	887	59.0	0.90
	2	1,600	85	529	614	986	61.7	0.83
	3	1,608	81	773	854	754	47.0	0.99
	4	1,630	95	750	845	785	48.2	0.87
	5	1,644	98	848	946	698	42.4	0.91
	6	1,642	132	689	821	821	50.0	1.00
Mean		1,604	94	688	782	822	51.4	0.92

TABLE XI-B—concl'd

Sample number		PHOSPHATES (P O ₅)					CaO retained/ P ₂ O ₅ retained	
		Intake mg	OUTPUT			BALANCE OR RETENTION		
			Urine mg	Fæces mg	Total mg	Mg		Percentage of intake
Group II	1	1,578	96	584	680	898	56.9	0.86
	2	1,700	141	526	667	1,033	60.7	0.78
	3	1,716	146	684	830	886	51.8	1.04
	4	1,746	132	720	852	894	51.2	1.07
	5	1,740	142	827	969	771	44.5	0.91
	6	1,762	179	912	1,091	671	38.2	1.25
Mean		1,707	139	709	848	859	50.6	0.99
Group III	1	252	18	178	196	56	22.2	8.68
	2	266	22	166	188	78	29.3	6.87
	3	250	32	253	285	—35		
	4	282	36	224	260	22	7.8	3.68
	5	249	33	286	319	—70		
	6	289	41	148	189	100	34.7	5.71
Mean		265	30	209	239	25	9.4	
Group IV	1	1,558	124	458	582	976	62.6	0.95
	2	1,598	137	467	604	994	62.3	0.98
	3	1,546	151	666	817	729	47.2	0.99
	4	1,620	128	788	916	704	43.6	0.82
	5	1,640	148	723	871	769	46.8	1.24
	6	1,678	215	749	964	714	42.6	0.90
Mean		1,607	151	642	792	814	50.9	0.98
Group V	1	302	17	130	147	155	51.3	4.48
	2	286	13	121	134	152	53.1	5.30
	3	282	14	54	68	214	75.7	2.34
	4	281	13	176	189	92	32.8	4.22
	5	260	17	274	291	—31		
	6	272	24	178	202	70	25.8	7.30
Mean		281	16	156	172	109	38.8	
Group VI	1	1,550	41	516	557	993	64.3	0.89
	2	1,694	63	552	615	1,079	63.8	0.99
	3	1,676	58	727	785	891	53.2	1.02
	4	1,676	38	902	940	736	43.9	1.04
	5	1,720	51	879	930	790	45.9	1.14
	6	1,680	136	812	948	732	43.6	1.32
Mean		1,666	65	731	796	870	52.4	1.07

TABLE XI-C

Sample number		MAGNESIA (MgO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
Group I	1	262	23	148	171	91	34.8
	2	280	29	174	203	77	27.5
	3	281	23	138	161	120	42.8
	4	284	50	170	220	64	22.5
	5	287	47	160	207	80	27.9
	6	287	48	147	195	92	32.0
Mean		280	37	156	193	87	31.3
Group II	1	275	9	162	171	104	37.9
	2	296	20	183	203	93	31.4
	3	298	18	154	172	126	42.4
	4	305	35	158	193	112	36.7
	5	304	36	180	216	88	29.0
	6	308	22	182	204	104	33.8
Mean		298	23	170	193	105	35.2
Group III	1	116	18	62	80	36	31.0
	2	123	26	65	91	32	26.0
	3	115	42	79	121	—6	
	4	130	52	85	137	—7	
	5	115	58	64	122	—7	
	6	133	50	59	109	24	18.0
Mean		122	41	69	110	12	9.8
Group IV	1	272	31	139	170	102	37.5
	2	279	15	153	168	111	39.8
	3	270	25	135	160	110	40.8
	4	283	31	174	205	78	27.7
	5	286	28	135	163	123	43.0
	6	293	29	174	203	90	30.8
Mean		281	27	152	178	102	36.6

TABLE XI-C—concl'd

		MAGNESIA (MgO)					
		Intake mg	OUTPUT			BALANCE OR RETENTION	
			Urine mg	Fæces mg	Total mg	Mg	Percentage of intake
Group V	1	139	11	46	57	82	59.0
	2	132	8	45	53	79	60.0
	3	130	14	152	166	36	
	4	122	19	64	83	39	32.0
	5	120	17	54	71	49	40.8
	6	125	25	57	82	43	34.5
	Mean	128	16	70	85	43	33.6
Group VI	1	270	12	126	138	132	49.0
	2	295	12	164	176	119	40.3
	3	293	16	135	151	142	48.5
	4		28	186	214	79	27.0
	5	300	32	159	191	109	36.3
	6	293	23	161	184	109	37.2
	Mean	291	205	155	176	115	39.7

TABLE XII

Group	Sample number	OUTPUT OF CaO IN		OUTPUT OF P ₂ O ₅ IN		OUTPUT OF MgO IN	
		Urine	Fæces	Urine	Fæces	Urine	Fæces
		Percentage		Percentage		Percentage	
I	1	0.046	13.90	0.048	5.82	0.015	1.59
	2	0.058	13.20	0.049	5.08	0.017	1.66
	3	0.045	13.66	0.037	7.26	0.010	1.30
	4	0.072	14.32	0.038	7.19	0.020	1.64
	5	0.064	15.40	0.035	8.40	0.017	1.59
	6	0.062	14.58	0.042	7.50	0.015	1.62
	Mean	0.058	14.18	0.042	6.89	0.016	1.57
II	1	0.040	14.18	0.059	5.79	0.006	1.60
	2	0.078	13.32	0.091	4.65	0.013	1.62
	3	0.042	14.58	0.063	6.96	0.008	1.56
	4	0.126	12.64	0.068	6.97	0.018	1.52
	5	0.120	14.90	0.065	8.50	0.016	1.74
	6	0.080	14.36	0.087	8.57	0.011	1.70
	Mean	0.081	14.00	0.072	6.91	0.012	1.62

TABLE XII—concl'd

Group	Sample number	OUTPUT OF CaO IN		OUTPUT OF P ₂ O ₅ IN		OUTPUT OF MgO IN	
		Urine	Fæces	Urine	Fæces	Urine	Fæces
		Percentage		Percentage		Percentage	
III	1	0·072	12 60	0 019	4 78	0 019	1 66
	2	0 068	11 00	0 016	4 00	0 019	1 58
	3	0·136	12 80	0 017	4 84	0·023	1·52
	4	0·188	13 10	0 017	4 35	0 025	1 66
	5	0 180	12 92	0 017	6 20	0 029	1 39
	6	0·101	9·44	0·018	3·75	0 022	1 50
Mean		0 124	11 98	0 017	4 65	0 023	1 55
IV	1	0·032	14 94	0 063	5 52	0·016	1 68
	2	0 036	13 36	0·067	5 01	0 008	1 64
	3	0·044	14 40	0·063	6 90	0·011	1 40
	4	0 107	14 54	0 059	7 51	0·014	1 66
	5	0 052	13 70	0·056	7 90	0 011	1·46
	6	0·135	14 70	0 095	7 53	0·013	1 74
Mean		0 068	14 27	0·067	6 73	0 012	1 60
V	1	0·254	12·80	0 032	4 30	0·021	1 50
	2	0·055	12 04	0·031	4 34	0 020	1 60
	3	0·282	13·58	0·028	4 10	0·029	1 44
	4	0·335	12 20	0 021	4 05	0·029	1 47
	5	0·222	13 54	0·024	6 65	0 024	1 32
	6	0 161	10·66	0 027	4·29	0 028	1 38
Mean		0·218	12 47	0 027	4 62	0 025	1 45
VI	1	0·025	14·12	0 034	5 53	0 010	1 36
	2	0·015	13 26	0 054	5 32	0 010	1 61
	3	0 037	14 02	0 043	7 10	0 012	1 32
	4	0·061	13 50	0·027	7 88	0 020	1 62
	5	0·019	15 34	0 032	8 70	0 020	1 58
	6	0 059	13 82	0 091	8·24	0·015	1 63
Mean		0 036	14 01	0 047	7 13	0·015	1 52

of phosphates was excreted in the urine and about 45 per cent in the fæces, while about 51 per cent was retained in the system As regards MgO, 31 per

cent of the intake was retained and the balance was excreted to the extent of about 13 and 56 per cent in the urine and the faeces respectively

When vitamin A concentrate was added to the *basal* diet *plus* sodium phosphate, the urinary excretion of CaO was not much affected and was on the average about 6.5 per cent of the intake. The excretion through the faeces and the percentage retention remained much the same as in Group I, viz., at about 60 and 34 per cent of the intake respectively. The excretions of phosphates also were much the same as in Group I, resulting in 51 per cent of the intake being retained. Practically the same observations as in Group I were noticed for the excretion of MgO.

A diet same as that of Group II but having no sodium phosphate added to it gave results differing much from the previous two groups. The urinary excretion of CaO was as high as 21.5 per cent of the intake, while the faecal excretion was about 49.5 per cent, the balance of about 29 per cent was retained in the system. Over 90 per cent of the phosphate taken in was excreted in the urine and the faeces, leaving a small margin of about 9.4 per cent to be retained in the system. The excretion of phosphates was at times so great as to occasion even a negative balance. Likewise in the excretion of MgO, there was often a negative balance, but the average total excretion for the entire period was 90 per cent of the intake, thus leaving a balance of about 10 per cent for retention.

The excretions and the retention of CaO by rats fed on a *basal* diet containing sodium phosphate in addition to both vitamins A and D were not very different from those of Groups I and II, the average excretions being 6.6 per cent of the intake in the urine, 59 per cent in the faeces and the balance, nearly 35 per cent, retained in the system. The phosphate excretions and retention, too, were much the same as in Groups I and II, the figures being 9.4 per cent of the intake through the kidneys, 40 per cent through the bowels and the balance about 51 per cent retained. Almost similar results as in Groups I and II were observed with MgO too, resulting in a retention of 36.6 per cent of the intake.

The excretion of CaO, MgO and P_2O_5 by rats in Group V, whose diet was the same as that of Group IV but without the sodium phosphate, was very different to those of Groups I, II and IV. Thus 12.1 per cent of the intake of CaO was excreted in the urine, about 41 per cent in the faeces while about 47 per cent retained in the system. There was very little excretion of phosphates in the urine, while about 39 per cent of the intake was retained in the system. A negative balance of phosphates was once observed. Similarly in MgO too, there was a retention about 34 per cent of the intake, negative balance occurred once.

Lastly, in rats fed on the *basal* diet *plus* sodium phosphate *plus* cod-liver oil (Group VI), the urinary excretion of CaO was very little, amounting only to 2.1 per cent of the intake, about 60 per cent was excreted in the faeces. The percentage retention of CaO was 38.2, a result approximating that in

Groups I, II and IV The figures for the excretion and retention of P_2O_5 agreed closely with those for Groups I, II and IV, the retention of P_2O_5 being 52.4 per cent of the intake. The results for MgO too were much the same as in Groups I, II and IV, the retention being 39.7 per cent of the intake.

The above results expressed as percentages of either the urine or the faeces bring out much more significantly the differences between the Groups I, II, IV and VI on the one hand, and Groups III and V on the other. A study of Table XII will show that the average percentage excretions of the CaO in the urines of Groups I, II, IV and VI were 0.058, 0.081, 0.068 and 0.036 respectively with a mean of 0.061 per cent, while the values for Groups III and V were 0.124 and 0.218 respectively with a mean of 0.171 per cent, which is nearly three times as much in animals receiving sodium phosphate in their diets. Though the latter two groups of animals got a sufficiency of vitamins, chiefly as vitamin A, the fact that their urines contained large amounts of calcium may render them liable to stone-formation. The percentages of faecal CaO did not differ so much, the figures for Groups I, II, IV and VI being about 14 per cent on the average, while those for Groups III and V being in the neighbourhood of 12 per cent. Conversely, the percentage urinary excretion of phosphates in Groups I, II, IV and VI were 0.042, 0.072, 0.067 and 0.047 respectively with a mean of 0.057 per cent, the corresponding values for Groups III and V being 0.017 and 0.027 with a mean of 0.022 per cent, which is approximately a third of that of the animals receiving sodium phosphate. Thus there seems to be an inverse relation between the urinary calcium and phosphates. This inverse relationship was not only observed in this series, but also in the previous two series of experiments. A similar observation was noticed by Meigs *et al* (1919) in their experiments on the 'Physiology of phosphorus and calcium metabolism of dairy cows'. Again, the percentage of P_2O_5 in the faeces of Groups I, II, IV and VI was on an average about 7 per cent, while that of Groups III and V was only about 4.6 per cent. Lastly, the average percentage of MgO excretion in the urines of Groups I, II, IV and VI was 0.014 while the corresponding value for Groups III and V was 0.024. The MgO content of the faeces in all the six groups was much the same, varying from 1.5 to 1.6 per cent.

The body-weights of the animals are given in Chart 6. Tables XIII, XIV and XV give the food consumption of the animals in the different groups, the weekly increase in body-weights, volume of urine voided and weight of faeces excreted, and the total excretion of chlorides and nitrogen in the urines.

General considerations

It is stated that calcium is excreted both by the kidney and the large intestine, the greater part by the latter route (Cushny, 1918, Husband *et al*, 1923). It is also stated that diets rich in calcium cause only a slight increase in the urinary excretion of calcium (Telfer *et al*, *vide infra*), but as will be at once

apparent from the results of the present investigation, these statements require qualification. Under normal conditions, practically all the calcium of the diet,

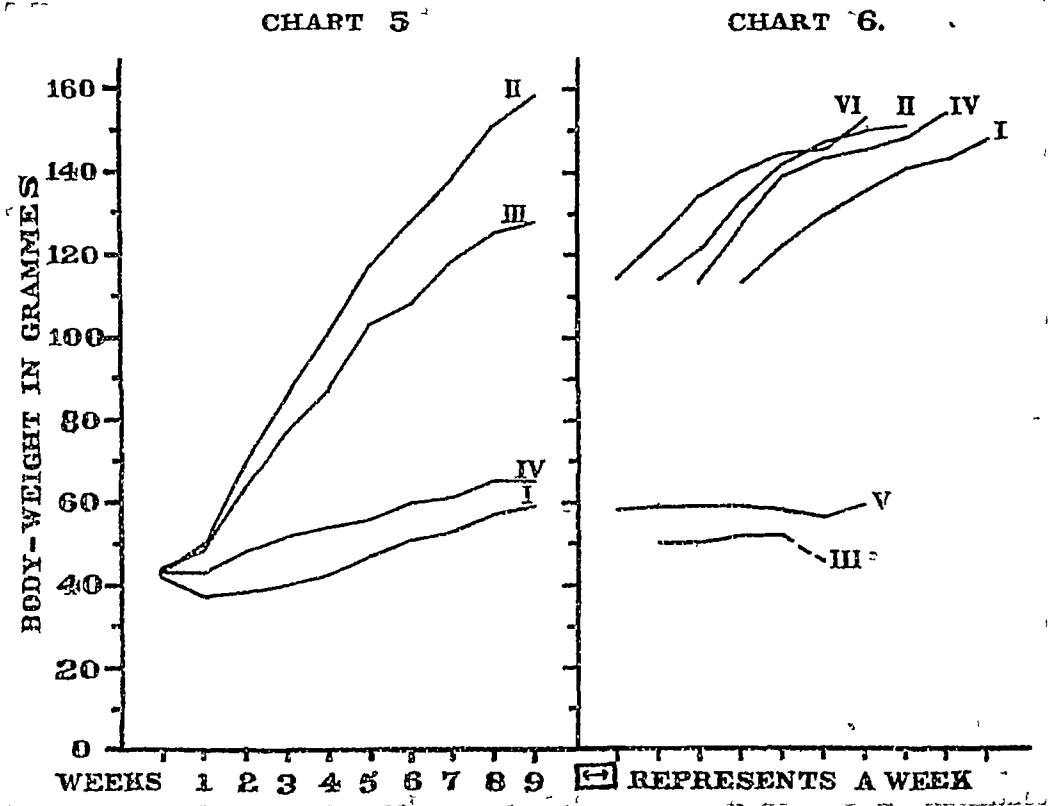


TABLE XIII

Showing food consumption

Sample number	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI
	Food gs	Food gs	Food gs	Food gs	Food gs	Food gs
1	294.3	309.5	159.7	304.8	180.1	304.5
2	314.4	333.1	159.1	313.9	171.6	331.7
3	314.8	335.3	149.4	303.2	168.6	329.2
4	319.1	343.0	169.2	317.4	168.2	328.6
5	322.6	340.7	149.3	321.2	155.8	337.0
6	321.5	345.2	172.8	328.5	162.1	330.0

TABLE XIV

Showing increase in body-weight, volume of urine voided and weight of faeces excreted

Group	Particulars	Sample number					
		1	2	3	4	5	6
I	Increase in body-weight, gs	18 0	13 0	12 0	13 0	4 0	9 0
	Urine voided, c c	154 0	174 0	220 0	254 0	282 0	318 0
	Faeces excreted (moist), gs	11 5	13 1	14 0	13 6	13 3	11 2
	Faeces excreted (dry), gs	9 28	10 42	10 65	10 36	10 05	9 07
II	Increase in body-weight, gs	13 0	25 0	17 0	11 0	5 0	3 0
	Urine voided, c c	162 0	155 0	232 0	194 0	220 0	206 0
	Faeces excreted (moist), gs	18 4	18 3	16 0	15 5	17 2	21 0
	Faeces excreted (dry), gs	10 12	11 28	9 84	10 37	10 33	10 67
III	Increase in body-weight, gs	0 0	3 0	1 0			
	Urine voided, c c	94 0	136 0	186 0	208 0	196 0	228 0
	Faeces excreted (moist), gs	6 1	5 5	7 6	6 5	6 1	5 5
	Faeces excreted (dry), gs	3 73	4 15	5 22	5 15	4 62	3 95
IV	Increase in body-weight, gs	27 0	24 0	6 0	7 0	6 0	11 0
	Urine voided, c c	198 0	205 0	240 0	218 0	266 0	226 0
	Faeces excreted (moist), gs	11 5	12 2	13 5	15 0	13 0	15 2
	Faeces excreted (dry), gs	8 30	9 33	9 63	10 45	9 18	9 93
V	Increase in body-weight, gs	2 0	1 0	0 0	-2 0	-5 0	+8 0
	Urine voided, c c	54 0	40 0	50 0	50 0	60 0	74 0
	Faeces excreted (moist), gs	4 7	3 3	4 8	5 1	4 6	5 4
	Faeces excreted (dry), gs	3 03	2 78	3 72	4 36	4 12	4 16
VI	Increase in body-weight, gs	20 0	21 0	13 0	7 0	5 0	13 0
	Urine voided, c c	121 0	117 0	136 0	142 0	160 0	150 0
	Faeces excreted (moist), gs	13 9	15 8	17 6	18 5	17 3	17 1
	Faeces excreted (dry), gs	9 32	10 19	10 22	11 46	10 06	9 87

excepting that actually utilized in the system, is excreted in the bowels. But in rats fed on diets deficient in fat-soluble vitamins and containing an excess of calcium, a considerable amount of calcium is excreted through the kidneys—an amount several times greater than that excreted by normally fed animals. Though the amounts excreted may not intrinsically be very great, yet in the light of the solubilities of salts like calcium hydroxide, phosphate, carbonate, etc., the significance of the values of urinary calcium becomes at once apparent, it is one of the positive factors predisposing the animals to stone-formation.

Deficiency of vitamin A is generally held to be one of the chief causes of stone-formation. Provision of an adequate amount of this vitamin does

TABLE XV

Showing urinary excretion of chlorides and nitrogen

Sample number	GROUP I		GROUP II		GROUP III		GROUP IV		GROUP V		GROUP VI	
	Chlorides gs	Nitrogen gs	Chlorides gs	Nitrogen gs	Chlorides gs	Nitrogen gs	Chlorides gs	Nitrogen gs	Chlorides gs	Nitrogen gs	Chlorides gs	Nitrogen gs
1	2.38	1.34	2.17	1.26	0.62	0.31	2.61	1.20	0.87	0.44	2.52	1.13
2	2.30	1.14	2.54	1.29	0.80	0.43	2.22	1.03	0.67	0.35	2.44	1.13
3	2.50	1.18	2.77	1.34	0.99	0.54	2.28	1.16	0.84	0.50	2.23	1.09
4	2.34	1.36	2.55	1.16	1.18	0.82	2.35	1.17	0.69	0.55	2.27	1.12
5	2.53	1.36	2.77	1.22	1.16	0.53	2.45	1.21	0.68	0.74	2.95	1.14
6	2.64	1.36	2.72	1.24	1.21	0.68	2.85	1.10	0.80	0.53	2.68	1.25

prevent stone-formation in rats fed on certain diets, but the results of the present investigation, wherein diets of such high stone-producing potency were used, indicate that mineral imbalance is a factor of even greater importance in the production of calcium carbonate or calcium hydroxide stones. It has been found in this investigation that the addition of fat-soluble vitamins to the diet, either as vitamin A alone or in association with vitamin D, did not materially alter the composition of the urine, in fact, the urinary excretion of calcium was much the same as when vitamins A or A and D were lacking. Yet the crucial point is whether such a state of affairs conduces to stone-formation in the urinary tract. From the values obtained of the urinary excretions of calcium on diets deficient in fat-soluble vitamins as also on diets containing them in sufficient amounts, the conclusion is irresistible that stone-formation is very likely. Nevertheless, not a single case of urinary calculi has so far been encountered in rats fed on diets adequate in vitamin A. There was the instance of a rat No. 3210, a male, getting the *basal diet plus* vitamin A concentrate (B D H) actually passing gravel in its urine. The discrete particles of gravel were found to be mostly of calcium hydroxide. Though most of the factors conducive to stone-formation were present in this case, yet, consequent on the presence of vitamin A in the diet, formed stones were not deposited in it. Hence as the particles of the calcium hydroxide formed, they were passed in the urine.

Again, these animals though receiving adequate amounts of vitamin A always run the risk of developing stones in their urinary tract during any inadvertent cessation of this vitamin. The results of the present investigation

suggest that this risk can be completely avoided or at least minimized to a great extent, by balancing the diet as regards its CaO and P_2O_5 contents. The balancing of the CaO and P_2O_5 contents of the diets is able to achieve much more than what adequate amounts of fat-soluble vitamins can achieve. The true significance of this statement is better understood when the growth of the animals is taken into consideration (*vide infra*). The effect of the slaked lime in rendering the animals liable to stone-formation can be counteracted by adding to the diets a phosphate in amounts equivalent to the calcium content of the diet to form the insoluble compound, calcium phosphate. The results of the present investigation show that not only stone-formation could be prevented by adding a phosphate to the *basal* diet but also that good growth was obtained approximating that obtained on the 'stock' diet of these laboratories. But by far the best approximation to the 'stock' diet was obtained when the *basal* diet contained, in addition to the phosphate, adequate amounts of the fat-soluble vitamins. It seems, therefore, highly necessary that a diet, if it is not to favour stone-formation of the calcium carbonate or calcium hydroxide variety should be well-balanced with respect to its mineral constituents, chiefly its calcium and phosphate contents. It also emerges from the foregoing experiments that the *basal* diet when so balanced with regards to its CaO and P_2O_5 contents, even though it remains deficient in fat-soluble vitamins is infinitely superior to the *basal* diet, ill-balanced as it is with respect to CaO and P_2O_5 but containing an adequate supply of the fat-soluble vitamins. As observed before, this difference in behaviour is better understood when the growth of the animals on the respective diets is considered.

Relation of calcium retention to phosphate retention.

Theoretically, on a normal diet, the ratio of retentions of CaO to P_2O_5 should be fairly constant. Supposing all the calcium were to be retained in the system as its phosphate, then the ratio, $\text{CaO retained}/\text{P}_2\text{O}_5 \text{ retained} = 168/142 = 1.18$. It should not be understood that this value is absolute, for all the calcium need not necessarily be retained as its phosphate alone. It has been found that the proportion of carbonate in normal rat bone increases with age (Kramer *et al*, 1928). Hence the ratio is bound to vary a little from the theoretical. A study of Tables I, VI and XI shows wide variations of the value, $\text{CaO retained}/\text{P}_2\text{O}_5 \text{ retained}$, with variations in the diet.

Thus with animals on the stock diet, the value was on the average 1.96, a figure rather too high from the theoretical. But when slaked lime was added to the above diet, the value was increased considerably, chiefly because of the greater elimination of phosphates. With the *basal* diet, the value averaged over 3. With radiostoleum added to the *basal* diet, the average for the ratio was 2.54, which was raised to 4.17 with the addition of milk. Without radiostoleum but with sesame oil added to the *basal* diet (Series I, Group IV), the average for the ratio was 3.05, while during the post-milk period on the same

diet, the value was higher still, and there was even a negative balance of phosphates in one week

In the second series of experiments, the average values for the ratio were 3.28 on the *basal* diet, and 2.37 on the *basal* diet to which milk was added. When sodium phosphate was added to the *basal* diet, the value was on the average only 0.92, but the *basal* diet *plus* butter gave as high a value as 3.38, a figure approximating closely that in the *basal* diet itself.

In the third series of experiments, the *basal* diet *plus* sodium phosphate gave a value of 0.92 for the ratio, *basal* diet *plus* sodium phosphate *plus* vitamin A concentrate 0.99, but *basal* diet *plus* vitamin A concentrate alone gave a much higher value, often leading to negative balance of phosphates. *Basal* diet *plus* sodium phosphate *plus* radiostoleum gave a value for the ratio 0.98, the same without the sodium phosphate (Group V) gave a far higher value, resulting in two out of 6 weeks in negative balance of phosphates. Finally, the *basal* diet *plus* sodium phosphate *plus* cod-liver oil gave a value 1.07, a result very close to the theoretical.

It is thus seen that out of thirteen different diets employed in the present metabolic studies, seven gave values approximating closely to the theoretical, while the remaining six gave far higher values ranging from over 3 to as much as nearly 40. It so happened that the animals on the former seven diets grew well, had good appetite and were free from disease, while those on the latter six diets suffered from loss of weight or did not grow at all, and ultimately died. It is also to be noted that the former seven diets on which good growth was obtained were those that were well-balanced with respect to their calcium and phosphate contents, whereas the latter six diets on which positively harmful and growth-inhibiting effects were observed were imbalanced as regards their calcium and phosphate contents. It is therefore obvious that a diet imbalanced with respect to its mineral constituents, although containing a sufficiency of the essential vitamins, is more harmful than a diet deficient in fat-soluble vitamins, but perfectly balanced as to its mineral constituents. Further, it emerges from the results of these experiments that comparatively little fat-soluble vitamins is needed to maintain good growth, if the diet is perfectly balanced with respect to its mineral constituents.

Compensating effect of vitamin D on mineral imbalance

Plimmer (1929) has stated that vitamin D possesses a compensating effect on the imbalance between the supply of calcium and phosphates. The foregoing metabolic experiments do not support this statement, for, if it were true, there ought to have been good growth in Group V, Series III, where the animals received adequate amounts of vitamin D. On the other hand, when the calcium and phosphate contents of the diet were well-balanced, good growth was obtained even when no extra vitamin D was added to it. The above results conclusively prove that vitamin A alone or in association with

vitamin D exercised no compensating effect on the imbalance between the supply of calcium and phosphates

Influence of calcium on the elimination of magnesium.

Cushny (1918) observes that 'the administration of calcium increases the elimination of magnesium in the urine' But Givens (1918) did not find a greater excretion of magnesium in the urine on ingestion of greater amounts of calcium. The results obtained in the preceding experiments do not support Cushny's observation but confirms that of Givens

Influence of fat on calcium retention.

Considerable difference of opinion exists as to the influence of fat on calcium retention, Stewart and Percival (1928) observe that 'it has often been stated that the absorption of calcium is greatly aided by the addition of fat to the diet, and equally often it has been denied that fat has any such effect' Mallon *et al* (1930) find that fat *per se* does not exercise a definite influence upon the calcium retention. In the present metabolic experiments detailed above, it was found that the calcium retention in animals receiving butter in as much as twenty per cent of their diet (Series II, Group IV) was not any greater than in those not receiving butter. In the same animals receiving butter, the amount of calcium retained was not appreciably different when the percentage of butter in the diet was gradually reduced from 20 to 10, 5 and finally 0, if at all, it was slightly more with reduced amounts of butter in the diet

Summary.

(1) The metabolism of calcium, magnesium and phosphate was studied in albino rats fed on thirteen different diets. Of these one was the 'stock' diet in use in these laboratories, but modified in certain regards. The remaining twelve were composed of white bread and yeast to which certain additions—milk, lime, butter, sesame oil, phosphates, vitamin A concentrate, radio-stoleum, cod-liver oil and combinations of these—were made. These diets included some that were deficient and some that were not deficient in fat-soluble vitamins, also some that were ill-balanced with respect to calcium and phosphates and some in which these mineral constituents were well-balanced.

(2) Very little calcium was excreted through the urinary tract of young rats in growing period fed on the 'stock' diet. Addition of excess of calcium salts to the 'stock' diet did not increase the urinary excretion of calcium.

(3) There was an abnormally great excretion of calcium through the kidneys of animals on diets deficient in fat-soluble vitamins and excessively rich in calcium. Provision of adequate amounts of these vitamins did not

decrease the urinary output of calcium. In normally fed animals, most of the calcium ingested was, apart from what was utilized in the system, excreted by way of the bowels, while in the deficiently-fed animals, a good percentage of the intake was excreted through the urine.

(4) There was a far greater excretion of phosphates in the urine of rats on the well-balanced 'stock' diet than in that of those fed on the deficient diets. But excess of calcium in the 'stock' diet decreased the phosphate-content of the urine.

(5) An inverse relation between the urinary calcium and phosphates was observed, more calcium was always associated with less phosphates, and vice versa.

(6) The necessity for balancing the diets with respect to their mineral constituents, chiefly calcium and phosphates, is indicated. The *basal* diet of white bread, yeast, lime and iodine, when correctly balanced as regards its CaO and P_2O_5 contents, was, though deficient in fat-soluble vitamins, far superior to the *basal* diet containing an adequacy of fat-soluble vitamins, but ill-balanced with respect to CaO and P_2O_5 .

(7) Fat-soluble vitamins, either vitamin A or in association with vitamin D, were not found to exercise a compensating effect on the imbalance between the supply of calcium and phosphates.

(8) Calcium carbonate and calcium hydroxide stones are not likely to arise on diets well-balanced with respect to their calcium and phosphate-contents, even though they may be deficient in fat-soluble vitamins. Provision of these vitamins in diets ill-balanced with respect to calcium and phosphates prevented the retention of calcium carbonate or hydroxide gravel in the urinary tract, but not its occurrence.

(9) The value for the ratio, CaO retained/ P_2O_5 retained, was near-about the theoretical in all animals receiving a balanced mineral ration, those receiving imbalanced diets gave values for the ratio far in excess of theoretical. Provision of fat-soluble vitamins had no effect on the value for the ratio when the imbalance between calcium and phosphate was not corrected.

(10) Animals receiving a balanced mineral ration, though deficient in fat-soluble vitamins, exhibited better growth, better health and better appetite than those receiving an imbalanced diet but provided with a sufficiency of fat-soluble vitamins. Nevertheless the growth, etc., was best when the fat-soluble vitamins were present in adequate amounts.

(11) Excess of calcium in the diet did not increase the elimination of magnesium in the urine.

(12) More chlorides and more nitrogen were eliminated in the urine of animals fed on a balanced mineral ration, than in that of those fed on ill-balanced mineral ration. Provision of fat-soluble vitamins did not affect the elimination of chlorides and nitrogen.

Conclusions.

The results of the present investigation bring out the importance of calcium-phosphorus imbalance in connection with the etiology of calcium carbonate and calcium hydroxide stones in albino rats. They also show conclusively that comparatively little fat-soluble vitamins is needed to maintain good growth, if the diet be perfectly balanced with respect to its mineral constituents. Provision of adequate amounts of fat-soluble vitamins did not improve the growth, if the imbalance between the mineral constituents was not corrected.

Further, a study of the values for the ratio $\text{CaO retained}/\text{P}_2\text{O}_5 \text{ retained}$ on the several diets investigated in the foregoing experiments suggests that this value may serve as a reliable criterion of the biological efficiency of a diet. Conformity of this value to the theoretical is a necessary pre-requisite to the efficient functioning of a diet. Its importance in devising a 'stock' diet for control animals employed in nutritional researches can hardly be over-estimated.

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RESEARCHES ON 'STONE'

Part X.

CATTLE STONE

BY

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[Received for publication, December 20, 1930]

PREVIOUS work on the chemical composition of urinary calculi in cattle (Ranganathan, 1931) has shown them to be of uniform chemical composition, consisting for the most part of calcium carbonate with a little magnesium, the latter presumably existing as the carbonate. It has also been shown that they contain very little nitrogen and no uric acid, further, that the chemical composition seems to be independent of the age of the animal and of the place of its nativity. As regards their physical characteristics, they are usually small, grain-like bodies, often little bigger than a No 4 lead-shot, having a light or deep golden-yellow sheen about them. They are also characterized by their fine, laminated structure and by the large numbers present in individual cases.

The present paper deals with a stone recently removed from the urethra of a six-months-old male calf, at Madura, which did not possess the physical and chemical characters of the 23 cattle stones previously reported upon (Ranganathan, 1931). It was single, about the size of a pea, was not laminated and had not the metallic sheen so commonly observed in cattle stones. In chemical composition, it also differed from those previously analysed. The following table shows its composition as also the average composition of a cattle stone.

	Moisture Per cent	AS PERCENTAGES ON MOISTURE-FREE SAMPLE					
		Nitrogen	P ₂ O ₅	CaO	MgO	CO ₂	Uric acid
No 25 (stone from calf)	16.7	7.6	22.0	10.9	12.5	0.00	0
Average composition of a cattle stone (representing the average of 23 stones)	3.1	0.37	0.90	44.0	4.84	39.07	0

In contrast to the previous 23 cattle stones, this one had a relatively high moisture content, high nitrogen, phosphates and magnesium content, and a relatively low calcium content, carbonates were not present in this stone. The nitrogen present in it did not exist as uric acid nor as an ammonium compound, but its nature could not be determined as there was not enough stone material for the necessary examinations.

The calf from which this stone was removed was fed on mother's milk for the first two months of its life, later it was fed on ground cotton-seeds (*Gossypium Sp*) with Bengal gram husk (*Cicer Arietinum*), rice bran and other household waste food materials.

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RESEARCHES ON 'STONE'

Part XI.

ON THE EFFECT OF MILK IN PREVENTING THE FORMATION OF CALCIUM STONES IN THE URINARY TRACT OF ALBINO RATS

BY

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[Received for publication, February 4, 1931]

IN a previous report (McCarrison, 1930) the influence of lime in favouring the production of urinary calculus in rats has been dealt with. It has been shown that the stones produced by means of a diet deficient in fat-soluble vitamins, in suitable protein and in phosphates, while at the same time excessively rich in lime, are of the calcium carbonate or calcium hydroxide variety or a combination of both.

The changes in calcium and phosphorus metabolism brought about in rats by this diet have likewise been discussed (Ranganathan, 1931) and emphasis laid on the lack of balance between calcium and phosphorus as an important factor in the causation of stones of this variety. Further, attention has been directed to the identity in composition and physical characters of the experimentally-produced calcium carbonate stones to those occurring under natural conditions in cattle (McCarrison, 1931).

The purpose of the present report is to record the effect of whole milk in preventing the development of calcium carbonate stones in albino rats.

The experimental diets.

The diets used in the present experiments were similar to those employed previously (McCarrison, 1930). But the concentration of lime in them was halved. Four diets were used; their composition is set out in Table I.

TABLE I

Showing the composition of the experimental diets

Diet	White bread, gs	Dried yeast, gs	Iodine solution drops	Lime grains	Milk, c c	Distilled water
I	97	3	25			<i>ad lib</i>
II	97	3	25		25	<i>ad lib</i>
III	97	3	25	12.5		<i>ad lib</i>
IV	97	3	25	12.5	25	<i>ad lib</i>

Twelve young rats—5 males and 7 females—aged between 30 and 40 days, and of body-weights ranging between 37 and 52 grammes, were fed on each of the first two diets. Sixty young rats—25 males and 35 females—of the same age, and of body-weights ranging between 36 and 53 grammes, were fed on each of the last two diets. Each animal was confined in a separate cage under conditions of scrupulous cleanliness. The experiments commenced on the 29th January, 1930, and were continued for 344 days.

Results of the experiments

The results are summarized in Table II.

TABLE II

Showing the results of the experiments

Description of diets	Number of rats	STONE IN THL			Cystitis	Dilated ureters	Pyonephrosis	Hydro-nephrosis
		Bladder	Ureter	Kidney				
I Without lime or milk	12	2	0	0	3	4	3	0
II With milk no lime	12	0	0	0	0	0	0	0
III With lime no milk	60	17	1	1	29	17	11	0
IV With lime and milk	60	0	0	0	3	1	1	0

From this table it is seen that the basal diet without lime or milk caused vesical calculus in 16.6 per cent, cystitis and dilated ureters in 25 per cent, and pyonephrosis in 33.3 per cent of the animals fed upon it. The addition of whole milk to this diet, in the proportion of approximately 5 c c per rat per day, completely prevented all these manifestations of disease in the urinary tract.

The addition of lime—1.5 to 2.5 grains of which were consumed by each rat daily—caused an increase in the incidence of vesical calculus of from 16.6 to 28.3 per cent and of cystitis from 25 to 48.3 per cent. The incidence of dilated ureters and of pyonephrosis was not significantly altered. The further addition of whole milk to this diet completely prevented the formation of urinary calculus but it did not, in the amounts consumed by the animals (approximately 5 cc per rat per day), wholly prevent the occurrence of inflammatory conditions in the tract, although greatly reducing their incidence. It is probable that in certain individual rats a larger quantity of milk was required to afford them complete protection.

As has been shown in previous reports (McCarrison, 1930, Ranganathan, 1931) the main faults in these diets, to which the formation of calcium carbonate stones are to be attributed, are (1) relative deficiency of phosphates and (2) deficiency of fat-soluble vitamins. To these must be added the general state of malnourishment resulting from the consumption of these diets. The addition of milk in sufficient quantity corrects all these faults.

Conclusion.

The addition of whole milk in sufficient quantity to a diet of white bread, yeast and lime completely prevents the formation of urinary calculi of the calcium carbonate or calcium hydroxide varieties in albino rats.

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RESEARCHES ON 'STONE'

Part XII.

ON THE RELATIVE IMPORTANCE OF VITAMIN A, RADIOSTOLEUM, COD-LIVER OIL AND SODIUM PHOSPHATE IN PREVENTING THE FORMATION OF CALCIUM STONES IN THE URINARY TRACT OF ALBINO RATS

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In a previous paper (McCarrison, 1931a) it is shown that the deposition of calcium carbonate or hydroxide or a mixture of both in the urinary tract of albino rats, fed on a diet composed of white bread, dried yeast, slaked lime and distilled water, was completely prevented by the addition of whole milk, in the proportion of 5 cc' per rat per day, to this diet. This amount of milk did not, however, wholly prevent the occurrence of inflammatory states of the tract (cystitis and pyonephrosis), and it was suspected that it failed to do so because it was insufficient for the varying needs of individual animals.

In view of our studies of calcium and phosphorus metabolism in relation to stone-formation (Ranganathan, 1931a) it was thought that whole milk exercised its protective action by virtue of its content of fat-solubles A and D or of its content of phosphates or of both. In order to test this presumption the experiments with which the present paper deals were undertaken.

The experimental diet.

The diet was that previously employed for the production of calcium carbonate and hydroxide stones (McCarrison, 1930*a*, 1931*b*) It consists of 97 parts of white bread, 3 parts of dried yeast, 25 drops of a solution of iodine (1 mg to the litre) in distilled water, and distilled water *ad libitum* To this diet slaked lime is added in varying proportions, in the present instance the amount was 5 grains per rat per day Various additions were made to this basal diet and groups of young rats were fed on the resultant mixtures, the experiments running concurrently The additions were as follows —

- 1 Vitamin A concentrate (B D H) in the proportion of one drop per rat per day
- 2 Radiostoleum (B D H)—containing both vitamin A and vitamin D—in the proportion of one drop per rat per day
- 3 Sodium phosphate, either with or without vitamin A concentrate or radiostoleum, in the proportion of 0.35* gramme per rat per day
- 4 Sodium phosphate as above together with cod-liver oil, in the proportion of 0.4 c.c. per rat per day

The experiments.

Sixty young rats, of body-weights ranging between 35 and 56 grammes, were used Of these 31 were females and 29 were males They were divided into 7 groups of the same mean body-weight, the sexes being as far as possible equally distributed in the various groups They were fed as follows —

- Group I On the basal diet containing slaked lime but without further additions
- Group II On the basal diet containing slaked lime together with sodium phosphate
- Group III On the basal diet containing slaked lime and sodium phosphate together with vitamin A concentrate
- Group IV On the basal diet containing slaked lime together with vitamin A concentrate but without sodium phosphate
- Group V On the basal diet containing slaked lime and sodium phosphate together with radiostoleum
- Group VI On the basal diet containing slaked lime together with radiostoleum but without sodium phosphate
- Group VII On the basal diet containing slaked lime and sodium phosphate together with cod-liver oil

Details of the experiments—the number of rats in each group, their mean initial and final body-weights, the average number of days they were under experiment, the mortality amongst them and the incidence of urinary calculi

* This amount was chosen as being that necessary to form, with the uncombined calcium ingested, insoluble calcium phosphate in the bowel

TABLE
Giving a summary of the experiments and their results

Group	No of rats in Group	Diet	Mean initial body-weight, gs	Mean final body-weight, gs	Average number of days under experiment	Mortality, per cent	STONE* IN THE			Cystitis	Dilated ureters	Pyonephrosis	Hydronephrosis	Hyperplasia of vesical mucous membrane	Hydrops testis
							Bladder	Ureter	Kidney						
I	12	B D = Basal Diet	40.5	62.5	139	83.3	6	0	0	4	3	2	0	0	0
II	12	B D + lime + Na_2HPO_4	40.5	140.4	214	41.6	1	0	0	1	0	0	0	4	3
III	12	B D + lime + Na_2HPO_4 + vitamin A concentrate	40.5	164.4	244	nil	0	0	0	0	0	0	0	0	0
IV	6	B D + lime + vitamin A concentrate	40.5	73.0	180	66.6	3	1	0	2	3	1	0	0	0
V	6	B D + lime + Na_2HPO_4 + radiostoleum	40.5	165.2	201	33.3	1	0	0	0	0	0	0	0	0
VI	6	B D + lime + radiostoleum	40.5	93.7	197	33.3	3	2	1	1	2	0	1	0	0
VII	6	B D + lime + Na_2HPO_4 + cod-liver oil	40.5	165.7	244	nil	0	0	0	0	0	0	0	0	0

* The stones varied in weight from 26 to 505 mg, that in Group V was the smallest

and of inflammatory states of the urinary tract, and certain other details—are set out in the accompanying table. The experiments were continued for 244 days when the survivors were killed.

Influence of sodium phosphate

The addition of sodium phosphate to the basal diet plus lime (Group II) had six notable effects: (1) it greatly improved the rate of growth of the animals, (2) it increased the average period of their survival and halved the mortality, (3) it greatly reduced the incidence of urinary calculus but did not wholly prevent the disease, (4) it reduced the incidence of inflammatory states of the urinary tract, (5) it caused a relatively high occurrence of hyperplasia of the vesical mucous membrane, and (6) it induced 'hydrops testis' (McCallison, 1930*b*) in 3 out of the 5 males in the group (Group II). Five animals died in this group: three from pneumonia, one from severe cystitis and the fifth from an unknown cause. That is to say, the relatively high mortality (41.6 per cent) was due in the main to bacterial infection of one kind or another. [With respect to the rate of growth of animals fed on the basal diet plus lime, wherein the calcium/phosphorus balance was properly adjusted by the addition of the requisite amount of sodium phosphate, it must be emphasized that any imperfect growth exhibited by them was due to disease acquired as a consequence of insufficiency of vitamin A in the diet. When no such disease occurred their rate of growth was as good as in animals receiving vitamin A concentrate or radiostoleum in addition to phosphate.]

Influence of vitamin A concentrate.

The addition of vitamin A concentrate to the basal diet plus lime (Group IV) did not improve the rate of growth of the animals to any marked extent, it prolonged the average period of their survival and lowered the mortality rate, it halved the incidence of stone (the case of ureteral stone in this group occurred in an animal which had a large collection of calcium carbonate calculi in the bladder), and, it halved the incidence of cystitis and pyonephrosis (compare with Group I). Four animals died in this group: three from vesical calculus and its sequelæ, the fourth from an undiscovered cause. It is significant that no animal in this group died as a result of pulmonary infection.

Influence of radiostoleum.

Radiostoleum (B D H) contains both vitamin A and vitamin D. Its addition to the basal diet plus lime (Group VI) caused a considerable improvement in the rate of growth of the animals, prolonged the average period of their survival, markedly reduced the mortality rate, and halved (approx.) the incidence of stone and its sequelæ (the two ureteral stones and the renal stone in this group occurred in animals which were suffering also from vesical

calculus) Two animals died in this group both as a result of stone and its sequelæ

Influence of vitamin A plus phosphate.

The addition of phosphate and vitamin A to the basal diet plus lime (Group III) greatly improved the rate of growth, lowered the mortality to nil and completely prevented the occurrence of stone and of inflammatory or other morbid states of the urinary tract. What phosphate alone or vitamin A alone could not achieve was achieved by the addition of both to the diet.

Influence of radiostoleum plus phosphate.

The addition of phosphate and radiostoleum to the basal diet plus lime (Group V) greatly improved the rate of growth of the animals, prolonged their average period of life and reduced the mortality rate to 33·3 per cent. But it did not afford the animals complete protection against stone. In these regards it was inferior to vitamin A concentrate, probably because the amount of vitamin A contained in the radiostoleum was too small for this purpose or for the purpose of protecting the rats against infection. Two animals died in this group, one from pneumonia (this being the animal in which vesical calculus occurred), the other from intestinal disease.

Influence of cod-liver oil plus phosphate.

The addition of cod-liver oil and phosphate to the basal diet plus lime greatly improved the rate of growth of the animals, reduced the mortality to nil and afforded them complete protection against stone and inflammatory or other morbid states of the urinary tract. Protection was also afforded against pulmonary and other infections. In these respects cod-liver oil plus phosphate had the same effects as vitamin A concentrate plus phosphate.

Influence of sex.

No significant sex differences regarding the incidence of stone or of its sequelæ were brought out by the results of these experiments.

Summary and conclusions.

It is evident from the above results that neither sodium phosphate alone nor vitamin A concentrate alone nor radiostoleum alone wholly prevented the deposition of calcium carbonate or hydroxide stones in the urinary tract of albino rats fed on the basal diet plus lime. But the addition of both phosphate and vitamin A, either in the form of cod-liver oil or of vitamin A concentrate, afforded the animals complete protection. The protective action of radiostoleum when added to the diet together with phosphate was not so great as that of either cod-liver oil or vitamin A concentrate.

There are two essential factors in the production of calcium carbonate stones in rats the first and most important, is a deficiency of phosphate relative to the amount of calcium ingested, the second is an insufficiency of vitamin A in the diet But a balanced adjustment between calcium and phosphorus will not wholly prevent stone unless the diet contains at the same time a sufficiency of vitamin A

The majority of urinary calculi in Indian cattle appear to be of the calcium carbonate variety and of a composition practically identical with those produced under experimental conditions in rats (Ranganathan, 1931*b*) It is reasonable, therefore, to conclude that their causes are the same in the two species of animals and to predict that a sufficiency of *green* fodder (to provide vitamin A) as part of a ration well balanced with respect to calcium and phosphates will prevent the occurrence of calcium carbonate stones in cattle

The results recorded in this paper are in conformity with the metabolic studies reported in another part of this Journal by the junior author (Ranganathan, 1931*a*)

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EXPERIMENTAL PRODUCTION OF GASTRIC ULCER IN ALBINO RATS

(A PRELIMINARY REPORT)

BY

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[Received for publication, February 4, 1931]

GASTRIC and duodenal ulcers are extremely common in Southern India so much so that at the General Hospital, Madras, the Medical Mission Hospital, South Travancore, and other hospitals in the south of India hundreds of surgical operations are performed annually for the relief of these conditions. The relative frequency of the two maladies is not definitely known, but gastric ulcer appears to be the more common in Madras, duodenal ulcer the more common in Travancore. Two surgeons in South India—Lieut-Colonel E W C Bradfield, IMS late Superintendent of the General Hospital, Madras, and Dr T H Somervell of the South Travancore Medical Mission—who have had a very large surgical experience of these two conditions have formed the opinion that their occurrence is in some way related to the diet of the people of these territories. Dr Somervell has undertaken a study of duodenal ulcer from this point of view, the results of which have convinced him that the tapioca diet in use by the people of South Travancore is definitely related, in a causative way, to the disease. This view has been submitted to the test of experiment, and the purpose of this preliminary report is to record the results of the test.

The experiments.

The animals used were albino rats taken from the healthy and well-fed stock of these laboratories. The freedom of this stock from gastric or duodenal ulcer was established at post-mortem examination of over 600 animals of all ages. The stock rats are fed on a diet consisting of whole wheat flour *chapa*

lightly smeared with fresh butter, the hard crusts of white loaves, sprouted Bengal gram, fresh raw cabbage and carrots *ad libitum*, whole milk, water, and a small ration of fresh raw meat with bones once every week or ten days. They live under perfect hygienic conditions, and are exposed daily to the sun's rays. In these circumstances neither gastric nor duodenal ulcer arises, nor, indeed, do any of the gastro-intestinal ailments so common in man.

The hygiene of the rats in the present experiments was equally good. They, too, lived under conditions of scrupulous cleanliness and were exposed daily to the sun's rays, but the conditions of the experiments required their separation from their fellows, while straw-bedding, which the stock animals enjoyed, was withheld from them because of the habit rats have of eating it. They were thus more exposed to 'chill' than the stock animals and this was, no doubt, a factor concerned in the frequent occurrence of pneumonia amongst them, this factor was, however, of secondary importance to food.

Two groups of 18 young rats, of body-weights ranging from 44 to 96 grammes, were fed on the two diets of which details are given below. The experiments, which ran concurrently, were continued for 675 days by which time most of the animals had died. Details regarding them are set out in Tables I and II.

The experimental diets

The staple article of diet of the people of South Travancore is tapioca, a food of low protein and fat-content and almost wholly lacking in the three important vitamins A, B and C. With this, and a certain amount of rice, fish and condiments, the poorer classes of the people seek to sustain themselves. In addition to its deficiencies in some directions and to its excesses in others, the diet is a very bulky one, a factor which has to be taken into consideration in evaluating its disease-producing potency. In August 1927, Dr. Somervell gave the following as the average diet of his last 30 cases of duodenal ulcer —

Morning meal

Tapioca	1 to 1.5 pounds
Red chillies (pepper)	30 to 40 grains
Tamarind	30 to 40 "
Rice water ('conjee')	1 to 1.5 pints

Midday meal

Tapioca	1 to 2 pounds
Red chillies (pepper)	30 to 40 grains
Tamarind	30 to 40 "
Rice water ('conjee')	2 pints

Evening meal

Tapioca	0.5 to 1 pound
Rice	1 to 2 pounds
Fish with ordinary curry-stuffs	(Amount not stated)
(Meat occasionally, chiefly at night)	

TABLE I (GROUP I)

[illegible]

'The tapioca is prepared for consumption in one or other of two ways —

- (a) The outer skin is cut from the fresh roots, the roots are boiled and the water discarded as soon as it comes to the boil, a little salt is added and the root is then eaten in its pulpy condition
- (b) The outer skin is cut off and the root dried for 4 or 5 days. It is then washed in cold water and boiled as before

The poorer people employ the first method almost exclusively. The root is always first soaked or boiled, as the people believe it contains a soluble poison' (Dr Someivell)

In the preparation of our experimental diets we employed, as far as possible, the methods of cooking in use by the people of Travancore. Two diets were used, the one including tapioca, the other not. Their composition was as follows —

The tapioca diet

Tapioca	.	10 oz
Rice		10 oz
Chillies		1/8 oz
Tamarind		1/8 oz
Fish		2 oz
Rice water		<i>ad libitum</i>

The rice diet

Rice	20 oz
Chillies			1/8 oz
Tamarind			1/8 oz
Fish			2 oz
Rice water			<i>ad libitum</i>

The rice used in the preparation of these diets was parboiled, milled and of fair quality.

Results of experiments

In each group one animal was excluded because of the short time it had lived on the diet. There remained 17 animals in each group which survived for 174 days or longer. These enabled a comparison of the two groups to be made and both to be contrasted with the stock animals, which, during the same period, remained free from disease —

- (1) The mortality in both groups was very high. 94 per cent in the group fed on the tapioca diet, and 82 per cent in the group fed on the rice diet.
- (2) In both pneumonia was the principal cause of death. The most conspicuous effect of the diet was, therefore, to render the animals highly susceptible to infection of the lungs. Other less common causes of death were anæmia, enteritis and inanition. In nine out

of the 30 animals dying during the course of the experiment the cause of death was not definitely ascertained

- (3) The growth of the animals in both groups was poor, in this respect the tapioca diet was slightly the better of the two
- (4) Eight out of 17 animals fed on the tapioca diet, or 47 per cent, were found at post-mortem examination to have lesions of the stomach and six out of 17 fed on the rice diet (or 35 per cent)
- (5) Those fed on the tapioca diet were more subject to congestion of the stomach and to gastritis both were liable to epithelial overgrowths at isolated areas in the proximal or squamous portion of the stomach and to ulceration of this part of the viscus Those fed on the tapioca diet were definitely more liable to ulceration of the distal or mucous portion of the stomach In neither did duodenal ulcer occur, though two of those fed on the 'tapioca diet' and one fed on the 'rice diet' were found at post-mortem examination to have duodenitis

These results are summarized in Tables I and II

The gastric ulcers

These were of small size measuring 0.1 to 0.5 cm in diameter They occurred both in the squamous and in the mucous portions of the viscus In the former locality they had raised edges which were often undermined, in the latter they were depressed below the surface of the mucous membrane They were best observed by opening the stomach and spreading it out on a glass plate after gently washing away adherent food-material In some cases blood clot was found lying free in the stomach, in others the mucous membrane was studded at various points with small clots, about the size of a small pin's head, which were tightly adherent and resisted removal It has not been possible to examine all these histologically but where such examination was made the area underlying the clot was found to be the seat of a small ulcer

Conclusion

Both the 'tapioca diet,' eaten by the people of South Travancore, and the 'rice diet,' which is similar in composition to that eaten by many people in the Madras Presidency, are capable of causing gastric ulcer in albino rats The tapioca diet is the worst of the two

URINARY EXCRETION OF IODINE BY GOITROUS AND NON-GOITROUS PERSONS IN GILGIT

Part II.

BY

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[Received for publication, March 12, 1931]

In a previous report (McCarrison *et al*, 1931) an account was given of the iodine-contents of the urines of goitrous and non-goitrous persons in Gilgit (McCarrison, 1906). It was found that no significant difference existed between them. The urines dealt with in that report were collected during the late spring and early summer months, and it was emphasized that the results observed related only to that season of the year.

The present report deals with the analyses of a further series of 45 urines—31 from goitrous and 14 from non-goitrous individuals—collected during the summer and early autumn months*. The results of the iodine-determinations are set out in Table I.

TABLE I

Showing the results of iodine-estimations in the urine of 31 goitrous and 14 non-goitrous persons in Gilgit, Kashmir iodine in γ per litre of urine

GOITROUS				NON-GOITROUS			
No	Sex	Age	Iodine	No	Sex	Age	Iodine
1	F	6	10	32	F	11	9
2	F	11	12				
3	F	14	8				
4	F	15	12				
5	F	20	11				
6	F	22	10				

*We are indebted to Major J C Pyper, IMS, Agency Surgeon, Gilgit, and to his staff for these samples.

TABLE I—*concl'd*

GOITROUS				NON GOITROUS			
No	Sex	Age	Iodine	No	Sex	Age	Iodine
7	F	24	10				
8	F	25	13				
9	M	26	6	33	M	26	6
10	M	26	5				
11	F	27	15	34	M	27	6
				35	M	28	4
12	M	29	7				
13	F	30	16	36	M	30	5
14	M	30	20				
15	M	32	12				
16	F	32	15				
17	M	34	5	37	M	34	4
18	M	35	5				
19	F	35	8				
20	F	36	10	38	F	36	14
21	F	36	16	39	F	36	4
22	F	37	14				—
23	M	40	5	40	M	40	22
24	F	40	6				
25	M	40	5				
26	M	40	12				
27	F	40	12				
				41	F	42	15
				42	M	42	7
				43	M	44	1
				44	M	44	5
28	F	46	10	45	F	46	8
29	M	46	6				
30	M	48	5				
31	M	50	8				

These results are shown as averages in Table II

TABLE II
Showing the results as averages

	No	Range of ages	Iodine in γ per litre
Goitrous both sexes	31	6—50	9.9
Non-goitrous both sexes	14	11—46	9.1
Goitrous females	18	6—46	11.6
Non goitrous females	4	11—46	11.5
Goitrous males	13	26—50	7.8
Non goitrous males	10	26—44	8.1

From the results set out in Tables I and II it is clear that no significant difference exists between the urinary excretion of iodine by goitrous and non-goitrous persons in Gilgit during the late summer and early autumn months

These results may be compared with those in the first series (McCarrison *et al*, 1931)

	1ST SERIES		2ND SERIES	
	No	Iodine in γ per litre	No	Iodine in γ per litre
Goitrous both sexes	36	7.1	31	9.9
Goitrous females	16	7.4	18	11.6
Goitrous males	20	6.9	13	7.8
Non goitrous both sexes	33	9.5	14	9.1
Non goitrous females	15	10.6	4	11.5
Non goitrous males	18	8.6	10	8.1

From this comparison it is seen that while goitrous individuals excrete slightly more iodine in the urine during the late summer and early autumn than during the late spring and early summer, the urinary excretion of iodine by non-goitrous persons is approximately the same at both seasons. A further result emerges from these findings: females, whether goitrous or non-goitrous, excrete slightly more iodine in the urine than males.

CONCLUSIONS

(1) There is no significant difference in the urinary excretion of iodine by goitrous and by non-goitrous persons in Gilgit. The conclusion reached in our first report is thus confirmed (McCarrison *et al*, 1931)

(2) The urinary excretion of iodine both by goitrous and by non-goitrous persons in Gilgit appears to be influenced, to a slight extent, by sex and by season

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Idem (*et alia*) (1931)

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Ind Jour Med Res, XVIII, 4, pp 1335-1346

IODINE-CONTENT OF THE THYROID GLAND IN GUINEA-PIGS FED ON A SCORBUTIC DIET

BY

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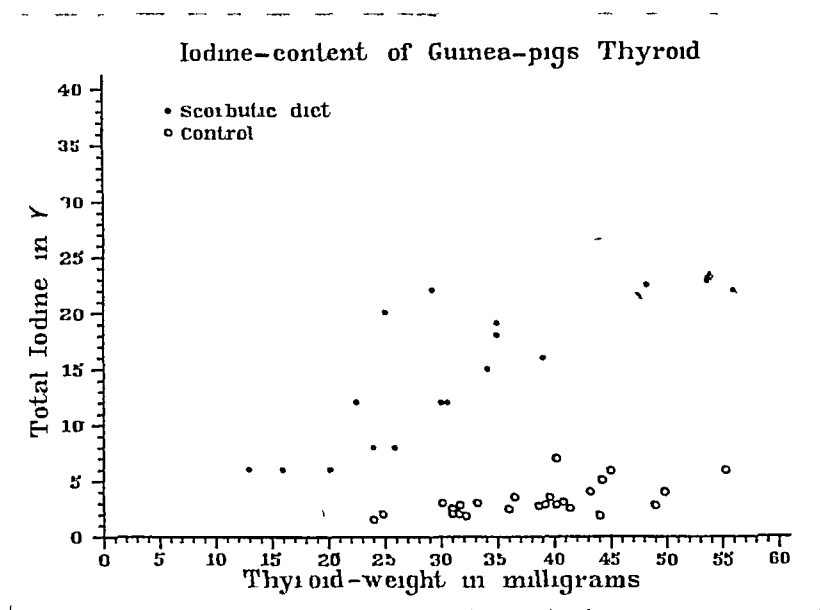
[Received for publication, February 9, 1931]

IN a previous paper (Sankaran, 1930) dealing with the iodine-content of the normal thyroid glands of albino rats, fed on a well-balanced stock diet, it was shown that the iodine-content of the gland varies directly with its weight. This positive correlation exists only in normal glands, in those that are hyperplastic the correlation is reversed the iodine-content being then in inverse relation to the size (weight) of the hyperplastic organ. This inverse relation is well illustrated by the goitres produced in rabbits by means of cabbage diets (McCarrison, 1931)

In all of Colonel McCarrison's recent work on the effects of faulty diets on the thyroid gland the iodine-content of the glands of animals fed on these diets has been estimated and compared with that in control animals of the same species fed on diets in which the various faults under study have been corrected

In the course of this work 25 guinea-pigs were fed on a scorbutic diet of crushed oats and autoclaved milk in the proportion of 50 grammes of the former to 25 grammes of the latter. A second group was fed on a well-balanced stock diet consisting of carrots, sprouted Bengal gram, fresh cabbage, green grass, bran and tap water. The iodine-content of these two diets was not estimated, but it is safe to assume, judging from the composition of the latter, that it was richer in iodine than the former. Each animal was confined in a separate cage under conditions of scrupulous cleanliness. Those fed on the scorbutic

diet rapidly developed signs of scurvy and died within 15 to 30 days, the control animals remained in good health. Nineteen thyroids from the former group and 25 from the latter were available for estimation of their iodine-content. The results of these estimations are set out in the accompanying Table I and spot diagram (Text-figure)



Text-figure

It is obvious from the diagram that the iodine-content of the thyroid glands of guinea-pigs fed on the scorbutic diet is significantly higher than in guinea-pigs fed on the well-balanced stock diet. Further, in both cases a high positive correlation exists between the iodine-content and the weight of the gland.

Confirmation of these results is afforded by the histological appearances of the gland in the two groups. These are indicated in Plate I, figs 1 and 2, which are representative specimens kindly prepared by Rai Sahib Mula Singh in the Pathological Department of these laboratories. It will be noted that the glands of guinea-pigs fed on the scorbutic diet are markedly richer in iodine-containing colloid than those of the control animals. It is not the purpose of this paper to go into histological details nor to attempt an explanation of this unexpected finding, these are matters which will be dealt with by Colonel McCarrison in another place. My purpose is merely to record the results observed.

PLATE I

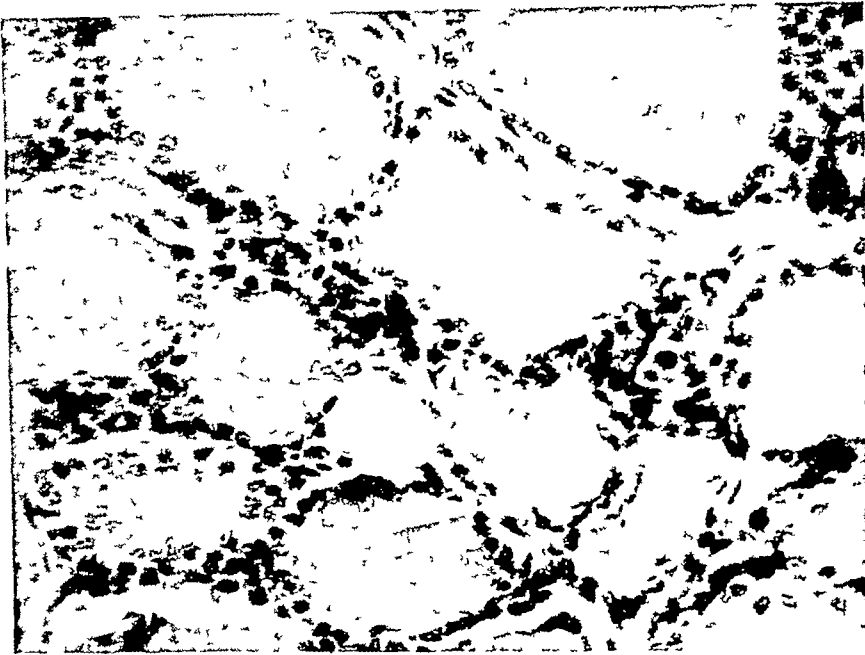


Fig 1—Thyroid gland of guinea-pig fed on scorbutic diet Note much larger content of colloid in the gland

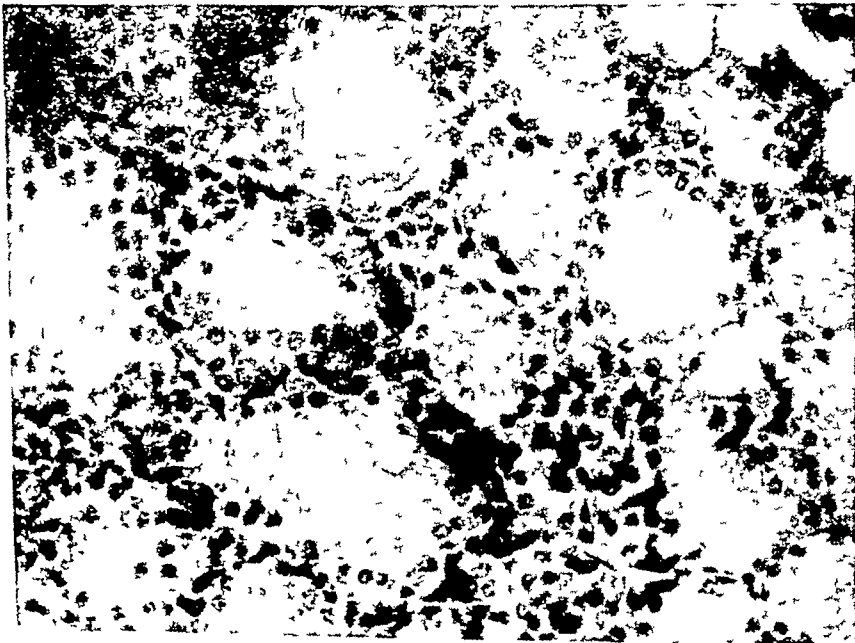


Fig 2—Thyroid gland of control guinea-pig fed on stock diet.

TABLE I

Showing iodine in guinea-pigs thyroids

SCORBUTIO DIET				CONTROL DIET			
No	Weight of thyroid in mg	Iodine found in γ	Iodine per 100 mg thyroid	No	Weight of thyroid in mg	Iodine found in γ	Iodine per 100 mg thyroid
1	20.2	6.0	29.8	26	41.4	2.5	6.0
4	30.0	12.0	40.0	27	24.0	1.5	6.2
5	24.0	8.0	33.4	28	33.2	3.0	9.0
7	22.4	12.0	53.5	29	43.2	4.0	9.3
8	16.0	6.0	37.5	30	30.2	3.0	9.9
10	52.8	28.0	53.2	31	31.6	2.0	6.3
11	30.6	12.0	39.2	32	40.8	3.0	7.3
12	34.2	18.0	52.7	33	40.2	2.8	7.0
13	34.0	15.0	44.0	34	49.8	4.0	8.0
14	13.0	6.0	46.0	35	44.0	1.8	4.1
16	29.2	22.0	75.5	36	31.0	2.0	6.5
17	25.8	8.0	31.0	37	39.2	2.8	7.1
18	25.0	20.0	80.0	38	32.2	1.8	5.6
19	12.2	7.0	57.8	39	49.0	2.8	5.7
20	34.8	19.0	54.6	40	36.0	2.5	6.9
21	28.0	10.0	35.8	41	38.6	2.8	7.2
22	55.8	22.0	39.4	42	24.8	2.0	8.1
23	39.0	16.0	41.0	43	31.6	2.8	8.9
25	48.2	22.5	46.6	44	36.4	3.5	9.6
				45	55.2	6.0	10.9
				46	45.0	6.0	13.3
				47	40.2	7.0	17.4
				48	39.6	3.0	7.6
				49	44.2	5.0	11.0
				50	31.0	2.5	8.1

For comparison with the results reached in guinea-pigs the iodine contents of the thyroid gland in other species of animals are set out in Table II

TABLE II

Showing the iodine-content of the thyroid gland in different species of animals

	Iodine in γ per 100 mg fresh thyroid gland	Authorities
Albino rat	18.0	Sankaran, 1930
Cattle	112.0	Marine and Lenhart, 1909
Sheep	69.0	„ „
Hog	88.0	„ „
Rabbits	23 to 50	Rowett Institute (unpublished)
„ (Coonoor)	2 to 4	Sankaran, 1931
Fowl	140 to 209	Rowett Institute (unpublished)
Guinea pigs (scorbutic)	47.5	Sankaran, 1931
Guinea pigs (normal)	8.4	„ „

Summary.

(1) The iodine-contents of 19 thyroid glands of guinea-pigs fed on a scorbutic diet and of 25 fed on a control diet have been estimated

(2) The iodine-content was found to be significantly higher in the guinea-pigs fed on the scorbutic diet

(3) A high positive correlation between the weight of the glands and their iodine-content was found to exist in both groups

REFERENCES

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McCARRISON (1931)

Ind Jour Med Res, XVIII, 2, pp 563-575
Ibid, 4, pp 1311-1334

THE BASAL METABOLISM OF SOUTH INDIAN WOMEN

BY

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[Received for publication, January 10, 1931]

THE basal metabolism of humans, that is, the heat production of the normal individual, lying quietly, awake, twelve hours after a meal, is accepted as a physiological constant in many laboratories and clinics. While it has been until recently a purely physiological measure, to-day the determination of basal metabolism has become an important supplement in the diagnosis and subsequent treatment of a number of endocrine disturbances, notably toxic goitre and myxoedema.

Measurements on normal individuals in various temperate countries show that the general level of basal metabolism is not materially different in these countries. When measurements were secured under tropical or sub-tropical conditions, evidence began to appear of a basal metabolism lower than the Aub-Du Bois(1) and the Harris-Benedict(2) standards for Americans. The evidence was by no means unequivocal, however. Thus, Eijkman's early study seemed to demonstrate that there is no difference in the metabolism of natives in Batavia, Europeans living in the tropics, and Europeans living at home(3). Later studies, especially the observations by Almeida in Brazil(4), suggested that the tropical climate has a marked depressing effect on metabolism, and

(1) AUB, J C, and DU BOIS, E F, *Arch Intern Med*, 1917, **19**, p 831

(2) HARRIS, J A, and BENEDICT, F G, *Carnegie Inst Wash Pub No* 279, 1919

(3) EIJKMAN, C, *Arch f d ges Physiol*, 1896, **64**, p 57, *ibid*, *Jour de Physiol et de Path gen*, 1921, **19**, p 33

(4) DE ALMEIDA, A O, *Jour de Physiol et de Path gen*, 1921, **18**, pp 713 and 958, *loc cit*, 1924, **22**, p 12

during the last few years the general trend of opinion of a number of contributions from tropical or sub-tropical localities has been that, so far at least as natives are concerned, there is a tendency for a lowered metabolism in the tropics. Browns and blacks in Jamaica, however, were found not to have an appreciably low metabolism(1), and Mayas in Yucatan seemingly have a somewhat higher metabolism than the North American standards(2). These facts, coupled with the observation that Chinese and Japanese women students living in the United States had a definitely low metabolism(3), stressed the possibility of a racial factor entering into the metabolic activity(4).

Under tropical conditions various factors must exert an influence upon vital activity. Obviously humans living in the tropics are subjected to climatic conditions entirely different from those obtaining in Europe and North America, usually to a much higher temperature and often to a much higher humidity. But judging from the findings of the different co-workers engaged in a co-operative research with the Nutrition Laboratory on the metabolism of races in the tropics it is by no means clear that temperature and humidity alone are the controlling factors. Under any climatic conditions the state of nutrition is known to affect metabolism(5). The character of the diet also, particularly if it is poor in protein(6), is commonly considered an important factor in establishing the level of vital activity. The size and configuration of the body also influence metabolism. With native races living in the tropics other common causes suggested to account for a lowered vital activity are early marriage, large families and poor hygienic conditions. Hence it is clear at the outset that a study of racial metabolism involves a simultaneous consideration of many factors.

The idea of a specific racial factor has been little emphasized. In 1925 it was noticed, in a study of some undergraduates at Mount Holyoke College, U S A, that the Chinese and Japanese women students had a definitely lower metabolism than North American women(7). In this case there were no differences in climate, diet, exercise and general college routine. Data for

(1) STEGGERDA, M, and BENEDICT, F G, *Amer Jour Physiol*, 1928, **85**, p 621

(2) WILLIAMS, G D, and BENEDICT, F G, *Amer Jour Physiol*, 1928, **85**, p 634

(3) MACLEOD, G, CROFTS, E E, and BENEDICT, F G, *Amer Jour Physiol*, 1925, **73**, p 449, *ibid*, *Proc Nat Acad Sci*, 1925, **11**, p 342

(4) A racial factor in birds has been definitely shown by Riddle and Benedict (See BENEDICT, F G, *Bull Soc Sci d'Hygiene Alimen*, 1929, **17**, p 325)

(5) BENEDICT, F G, MILES, W R, ROTH, P, and SMITH, H M, *Carnegie Inst Wash Pub No 280*, 1919, *ibid*, *Proc Nat Acad Sci*, 1918, **4**, p 149, BENEDICT, F G, *Jour Roy Army Med Corps*, August, 1918, BENEDICT, F G, *Proc Amer Philos Soc*, 1918, **57**, p 479, BENEDICT, F G, *Bull Soc Sci d'Hygiene Alimen*, 1918, **6**, p 422

(6) Wang finds that rather sudden but marked changes in the protein intake are without significant influence upon basal metabolism (See WANG, C C, HAWKS, J E, HUDDLESTON, B, WOOD, A A, and SMITH, E A, *Journal of Nutrition*, 1930, **3**, p 79)

(7) MACLEOD, G, CROFTS, E E, and BENEDICT, F G, *Amer Jour Physiol*, 1925, **73**, p 419, *ibid*, *Proc Nat Acad Sci*, 1925 **11**, p 342

comparison were drawn from the large mass of normal material in North American studies, particularly in the Harris-Benedict series where a large proportion of the women upon whom measurements were made were of college age and were attending college. The difference in metabolism between these Chinese and Japanese women and the North American standards was so striking as to be suggestive of a clear racial difference. A number of other observations made in China and elsewhere(1) at about this same time seemed to confirm the view that there is a specific racial difference, although most of the studies were so conducted that it was difficult to separate clearly the climatic factor and the possible racial factor.

GENERAL PLAN OF RESEARCH

In comparing the metabolism of Orientals and other races with that of Americans and Europeans, the measurements should be made, in so far as possible, in order to determine the influence of the racial factor alone. Such measurements can be obtained only by studying Orientals who are living in the same country as the western race with whom they are to be compared, in which case the climate and the diet would be strikingly different from their own, or by studying westerners under tropical conditions and comparing their metabolism directly with that of the inhabitants of the tropics. The number of Orientals living in western countries that could serve as subjects for physiological observations is so small that the problem could never be settled adequately by a study of these alone. For this reason it becomes essential to carry out the observations directly in the tropics. Such studies can be made in two ways. They can be carried out in institutions where there are moderately well-equipped laboratories where respiration apparatus of an approved type can be installed and well-trained technicians can secure the records, or they can be carried out actually in the field in connection with archaeological, geological, and geographical expeditions. Here the situation is infinitely more complicated but by no means insurmountable.

For the purpose of determining whether race *per se* is a definite factor influencing metabolism, the Carnegie Nutrition Laboratory has engaged upon an extensive survey of the metabolism of various races, undertaken in co-operation with research workers in different parts of the world, both in the field and in physiological laboratories. It is hoped that ultimately a sufficient number of observations with different races will be secured to permit of an extended generalized discussion of the racial factor. As a part of this comprehensive investigation a co-operative research is being carried out by the

(1) BLUNT, K and DYER, M, *Jour Biol Chem*, 1921, **47**, p 69, EARLE, H G, *The Caduceus*, 1922, **1**, p 85, EARLE, H G, *Chinese Jour Physiol*, 1928, Report Series, No 1, p 59, TAKAHIRA, KITAGAWA, ISHIBASHI, and KAIANO, Report of Imperial Nutrition Institute, Tokyo, Japan, 1924, p 88, HINDMARSH, E M, *Australian Jour Exper Biol and Med Sci* 1927, **4**, p 225

Carnegie Nutrition Laboratory and the Women's Christian College, Madras, in which the basal metabolism of South Indian women is being studied. It is recognized that all of the factors discussed above as affecting life in the tropics are intimately interwoven in the measurements secured and that the time is not yet appropriate for making final comparisons between Indian women and women of other races. The data presented in this report are therefore given as a contribution primarily to the study of the physiology of Indian women, with special reference to their basal metabolism.

All the observations recorded in this report were made in the Department of Zoology and Physiology at the Women's Christian College, Madras, and were confined to women. This has a certain disadvantage, because women apparently have a much more variable metabolism than do men and the problem with women is therefore more complicated. But one might reasonably argue that if a careful survey is made with Indian women, it will be fair to assume that in comparison with western standards Indian men will show essentially the same picture.

Our experimental programme has three main divisions, (1) a study of the basal metabolism of South Indian women, (2) a study of the basal metabolism of western women living in India, and (3) a study of the acclimatization of western and Indian women when they change their place of abode from one country to another of different climate. These problems call for a much longer study than has thus far been made, and in this preliminary report only the information regarding the first part of the experimental plan will be presented.

We wish here to express our appreciation of the co-operation and financial support accorded to us by the Women's Christian College, Madras, and of the interest and encouragement of the Principal, Dr Eleanor McDougall.

METABOLISM STUDIES ON INDIANS BY OTHER INVESTIGATORS

A large number of metabolism studies have been made with the Chinese, Japanese, Australians, Mexicans, and Philipinos but relatively few measurements have been made of the basal metabolism of Indians. Mukherjee(1), in 1926, reported the results of observations on fifteen male Bengalis from 22 to 27 years of age. His measurements, which were carried out with the Douglas bag and Haldane gas-analysis apparatus, were made after the subjects had been without food for from 16 to 18 hours, at a room temperature averaging 27°C. The metabolism on the average was 9 per cent below the western standards. In a second paper Mukherjee and Gupta(2) report a new series on eighteen healthy Bengali men from 20 to 29 years of age, whose metabolism averaged 13.3 per cent below the Du Bois standards with individual variations

(1) MUKHERJEE, H. N. (1926) *Calcutta Med Jour*, **20**, p. 425

(2) MUKHERJEE, H. N., and GUPTA, P. C. (1931) *Ind Jour Med Res*, **18**, 3, p. 807

of from -0.3 to -31.2 per cent Sokhey(1), in a note to the Far Eastern Association of Tropical Medicine, gives results on male medical students in Bombay, presumably Marathas, ranging in age from 20 to 30 years. Using the Tissot gasometer, he found the metabolism to average 12 per cent below the Du Bois standards. Detailed data are not given.

Since this report deals exclusively with the metabolism of Indian women and not with the metabolism in the tropics in general, references are not given here to the somewhat extensive literature on this latter subject. By far the most complete and critical analysis of the literature on metabolism in the tropics, up to 1929, has been published by Teding van Berkhout(2).

TECHNIQUE

For these co-operative researches on racial metabolism a special apparatus was designed for use during actual field work, when conditions for metabolism observations are at best difficult. Obviously, much more satisfactory measurements could be made when the studies were carried out under the auspices of a well-established laboratory or clinic, where more refined apparatus could be installed. The modern method of measuring the basal metabolism by the graphic registration of the oxygen consumption has many advantages, chief of which is, in a research of this kind, that the record is permanent, objective, and uninfluenced by the operator. Although the other investigators co-operating with the Nutrition Laboratory in its racial research have used, without exception, the specially devised 'field respiration apparatus(3), on account of the advantageous location of the laboratory in Madras it was thought practicable to use a respiration apparatus enabling the graphic registration of the oxygen consumption(4).

The metabolism measurements were therefore made with a spirometer type of respiration apparatus, which was lent to Madras by the Nutrition Laboratory in Boston. In this apparatus a blower relieves the lungs of the work of circulating the ventilating air current, and the carbon dioxide produced by the subject is collected in a soda-lime bottle outside the spirometer. The accuracy of this apparatus was controlled in the Nutrition Laboratory by alcohol check experiments, and the thermometers and the barometer used with it were also carefully calibrated. After its arrival in Madras alcohol check

(1) SOKHEY, S. S., *Trans 7th Congress Far Eastern Assoc Trop Med*, Calcutta, 1927, 3, p. 321.

(2) VAN BERKHOUT, P. J. TEDING, *Mededeelingen van den Dienst d Volksgezondheid in Ned-Indie*, 1929.

(3) BENEDICT, F. G., *Boston Med and Surg Jour*, 1927, **197**, p. 1161, *ibid*, *Chinese Jour Physiol*, 1928, Report Series No. 1, p. 39, *ibid*, Abderhalden's *Handb d biolog Arbeitsmethoden*, 1929, Abt. IV, Teil 13, p. 1.

(4) BENEDICT, F. G., *Boston Med and Surg Jour*, 1925, **193**, p. 807.

experiments were again made and most satisfactory controls were also established there(1) Thus we have every confidence in the accuracy of the apparatus as a physical instrument

The method was used, tested by long experience, of taking readings each minute of the position of the spirometer bell, as indicated by the pointer carried on the counterpoise. These readings for ten consecutive minutes were plotted against the number of minutes and a straight line was drawn to represent the *average trend* of the plotted points. The difference in the spirometer level between the beginning and the end of the 10-minute period, as indicated by this line, was used in calculating the oxygen consumption per minute. Graphic tracings were also made regularly on a spring kymograph, without time record, and have proved of great help in observing the course of individual experiments and in the study of the respiration.

In this co-operative research the operator making the metabolism measurements (E D M) received her training in the technique at the Nutrition Laboratory

SUBJECTS USED AND MEASUREMENTS MADE

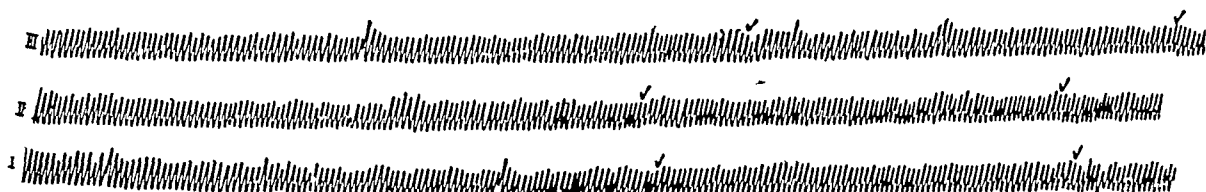
The Indian subjects were almost all resident students or members of the teaching staff of the Women's Christian College, Madras, whom we take this opportunity of thanking for their friendly interest and willing co-operation. They represent almost exclusively three groups, including 27 Tamils, 17 Malayalis, and 6 Telugus. In addition, there were two Coorgs and two Kanarese.

During the first few weeks of the research each subject came to the laboratory the day before her series of experiments was started, to see the apparatus and have it explained to her and, in a few cases, to make a preliminary trial. Later, when the experiments had become a topic of general conversation among the students and assurances from former subjects were abundant that there was no occasion for alarm, the preliminary meeting was dispensed with, with no apparent effect on the success of the experiment.

Menstrual days were avoided in all the measurements but a record was kept of the time of preceding menstrual period and a special study may be made later of the influence of this factor. The subjects came to the laboratory at 7 a.m., without choti-hazri and 12 hours after the last meal and lay on a bed for at least a half-hour, during which time room and mouth temperatures were taken, and data regarding the subject's personal history were collected. Both for the convenience of the operator and to avoid any possible disturbance of an exciting nature during the experiments, heart rates were taken with a stethoscope which was kept in place throughout the experimental period with a long lead to the ear pieces on the operator, out of vision of the subject.

(1) We acknowledge with appreciation the assistance of Mr. Edward Barnes, Professor of Chemistry at the Madras Christian College, in making these control experiments.

It is the general feeling of the foreign members of the staff at the College that Indian students are more excitable under unusual circumstances than English and American students in western countries. Accordingly we had anticipated considerable difficulty in securing a state of repose on the part of our Indian subjects during the experiments. Contrary to expectations, however, there was distinctly less difficulty in this respect than with the western women. With few exceptions the Indian women have adjusted themselves to the experiment with the greatest ease and quiet, the pulse has been steady and the respiration natural and regular. The respiration records secured were satisfactory in all the experiments. The figure shows a typical record, with shallow, fairly rapid, but quiet and regular breathing. The space between the two check marks, when the routine test for leaks was being made, represents a period of about four minutes.



Text-figure—Spirometer records of the respirations of a Tamil woman during three periods of measurement of the oxygen consumption under basal conditions

The check marks on each curve indicate when the weight was on the spirometer bell to test for leaks

The experimental periods for the measurement of the oxygen consumption were ten minutes each, and three periods were run on each of two consecutive or nearly consecutive days. In cases where any considerable discrepancy appeared between the measurements made on two days, the subject came again for further tests. On the average, the wet bulb thermometer registered 25°C and the dry bulb thermometer 27°C , with variations for the most part of from 2°C to 3°C . In a few instances wet bulb temperatures as low as 21°C with dry bulb temperatures from 1°C to 4°C higher were noted, and in a few other instances dry bulb temperatures as high as 31.5°C occurred with wet bulb temperatures of 25.0°C or 25.5°C .

Measurements of standing and sitting height, weight, vital capacity, and blood-pressure were made usually immediately after the metabolism experiments.

DISCUSSION OF RESULTS

Physical characteristics of the subjects

Inasmuch as height, weight, age, sex, physical configuration, and proportion of muscle to fat may influence metabolism, a careful study of the physical characteristics of our subjects is necessary. In Table I are recorded the body weight (without clothes) of each subject, the standing height (barefoot) the

TABLE I.
Physical characteristics of subjects

Subject number	Age yrs.	Body-weight (without clothes) kg	Height cm.	Sitting height cm	Pelvis	Subject number	Age yrs.	Body-weight (without clothes) kg	Height cm	Sitting height cm	Pelvis
Malayalis											
1	17	40.8	155	78.0	95	28	17	49.4	161	80.0	99
2	18	44.1	146	75.0	102	29	17	48.2	160	80.0	98
3	18	43.3	154	79.5	95	30	17	50.1	153	78.0	102
4	18	41.0	161	80.5	92	31	18	44.4	149	76.0	100
5	18	43.0	157	80.0	94	32	18	45.4	159	77.5	99
6	19	43.7	155	73.5	103	33	19	47.5	150	81.0	96
7	19	39.7	153	80.0	92	34	20	50.4	158	77.5	102
8	19	48.6	152	80.0	98	35	20	45.2	147	73.0	105
9	19	40.5	145	77.0	96	36	20	38.1	153	79.0	92
10	19	40.8	155	79.0	94	37	20	44.7	154	81.5	93
11	19	46.5	155	77.1	100	38	20	48.5	163	81.0	97
12	20	41.5	156	81.0	92	39	21	46.4	157	81.0	96
13	20	38.1	151	75.2	97	40	21	45.8	153	76.5	100
14	21	46.2	153	77.2	101	41	24	48.8	167	81.5	96
15	21	49.9	153	78.5	100	42	24	49.4	160	85.0	93

16	21	41 2	150	75 0	99	43	26	33 4	152	79 8	87
17	21	43 0	148	76 0	99	44	28	41 9	153	79 5	94
18	21	42 0	148	77 0	97	Average		45 7	156	79 3	97
19	21	37 2	153	79 0	91	Telugus					
20	22	62 1	166	86 5	98	45	18	40 6	148	73 0	101
21	22	57 2	161	82 5	100	46	21	40 6	151	79 5	93
22	22	43 2	153	77 5	97	47	23	46 7	156	78 5	98
23	23	41 8	154	78 0	96	48	23	47 5	152	81 0	96
24	26	39 6	158	81 8	90	49	24	44 0	152	77 8	98
25	28	44 8	145	74 0	103	50	24	41 6	154	75 5	98
26	30	67 6	155	78 5	112	Average		43 5	153	77 6	97
27	31	45 5	153	74 5	103	Coorgs					
Average		21	44 9	154	78 2	98	Coorgs				
						61	20	44 4	161	80 0	95
						52	23	45 2	153	79 3	97
						Kanarese					
						53	17	40 9	153	77 5	95
						54	27	50 6	153	78 0	102

sitting height, and the pelidisi or the index of the state of nutrition (computed from the sitting height and the weight) The subjects have been assigned numbers and the data for each group are given in accordance with increasing age

Age—The subjects range from 17 to 31 years averaging 21

Weight—The body weights as a whole were slightly low, judging from western standards, averaging for the Tamils 44.9 kg, for the Malayalis 45.7 kg, and for the smaller group of Telugus 43.5 kg There is an extraordinary uniformity in the weights of the different groups, with but few extremes Thus the heaviest woman is No. 26, weighing 67.6 kg, and the lightest No. 43, weighing 33.4 kg, but for the most part the weights range closely around the general average figure of 45 kg For comparison with westerners one should consider only the weights of similar age groups For purposes of this discussion we may refer to the weights for age and height given in the extensive tables of the Medico-Actuarial Mortality Investigation(1) and more specifically the adaptation of these weights made by Joslin(2) According to Joslin's adaptation of these data the average American woman having the same age and height as the average of our group of Tamils, namely 21 years and 154 cm, would weigh (without clothes) 48.5 kg The average weight of our group of Tamils is 44.9 kg or 3.6 kg less Thus, this group of Tamils and similarly the other groups on the average would seem to be slightly underweight for height and age when compared to American standards It would therefore seem necessary in discussing weight to take into consideration also the height and especially the sitting height

Sitting height—The sitting heights of our subjects were actually measured in every instance It is commonly assumed that the sitting height, when measured in accordance with all precautions especially emphasized by Dreyer(3), represents, with westerners at least, one-half of the total height

(1) Medico-Actuarial Mortality Investigation, compiled and published by Assoc. Life Insurance Medical Directors and The Actuarial Society of America, New York, 1912, 1, Table IX, p. 67

(2) JOSLIN, E. P., 'Treatment of Diabetes Mellitus,' New York, 1928, 4th ed., p. 967, *ibid.*, 'A diabetic manual for the mutual use of doctor and patient,' Philadelphia and New York, 1919, 2nd ed., Table XXXVII, p. 116

(3) DREYER (See DREYER, G., and HANSON, G. F., 'The assessment of physical fitness,' New York, 1921, pp. 5 and 6) states that the subject should sit on the floor and not hang the legs over a chair or bench He specifies that the measurements should be made as follows 'The subject places the backs of the fingers upon the platform on which he sits, and, with the fingers pointing backwards and the knees flexed, lifts the lower portion of the body gently backwards until the lowest bony portion of the os sacrum is in contact with the front of the measuring standard The back is then straightened until the back of the head comes into contact with the standard It will be found that different persons require to bend the knees in different degrees in order to achieve this position The head should be tilted neither up nor down and the eyes should look straight forward The measurement thus obtained gives the distance between the ischial tuberosities and the top of the head'

plus 5 cm. With the Tamils one-half of the average total height would be 77 cm and the actually measured sitting height is, on the average, only a little over 1 cm greater. Much the same picture is shown by the other groups. It would thus appear as if, judged from these measurements alone, these Indian women have relatively shorter stem lengths and longer legs than westerners.

Pelidisi—The connection between the state of nutrition and the vital activity or the metabolism of an individual is so close that with any group or race of people being studied one should secure the best possible index of the 'state of nutrition'. Of the various indices of the state of nutrition in use, the one that the Nutrition Laboratory has found most satisfactory for western subjects at least is the *pelidisi*, originated by the late Professor Clemens Pirquet of Vienna. The *pelidisi* is the relationship between the sitting height and the weight, expressed by the following formula: the cube root of ten times the weight in grammes divided by the sitting height in centimetres. For westerners Pirquet has made an elaborate study of the *pelidisi* and has prepared a series of tables from which, knowing the sitting height and the weight, one can derive the *pelidisi* directly (1). In his original preparation of the material Pirquet assumed that for small children a *pelidisi* of 100 represents the ideal. It has since been found that for adults a value somewhat less than this, probably nearer 97 or 98, represents the normal state of nutrition. Whatever opinion one has with regard to the quantitative significance of the *pelidisi*, one can assume with westerners at least that a *pelidisi* around 90 represents a distinctly low state of nutrition and that a *pelidisi* over 100 (sometimes values as high as 112 and 113 have been found) usually represents a fat person. We have found this index extremely helpful in the interpretation of the configuration of individuals.

It is of interest to determine whether the *pelidisi* can be used properly as an index of the state of nutrition of Indian women and compared with the *pelidisi* as found with normal western women. The average *pelidisi* of the 103 western women studied by Harris and Benedict is 96. As will be seen from Table I, the average *pelidisi* of our group of Tamils is 98, with individual variations ranging from a minimum of 90 with subject No. 24 to a maximum of 112 with subject No. 26. With the Malayals the average *pelidisi* is 97, but the minimum is as low as 87 in the case of subject No. 43 and the maximum is only 105 with subject No. 35. The values for the Telugus and the other groups lie closely around the average of 97. With the Tamils there is no instance of a *pelidisi* below 90, only one value of 90 and one of 91. There are three values of 92, two of 94, and two of 95. The rest are all higher. From this evidence alone, therefore, one could not conclude that these women were appreciably under-nourished. The average *pelidisi* of 98 would indicate

(1) Pirquet, C., 'System der Ernährung,' Berlin, Vols 1-4, 1917-1920 (also *Zeitschrift für Kinderheilk*, 1916-1918, Vols 14-18), *ibid*, 'An outline of the Pirquet system of nutrition,' Philadelphia and London, 1922, pp. 90, *et seq*.

a normal individual, although the average weight of the group of Tamils as a whole is nearly 4 kg less than the standard for western women of the same age and height (*see above*) Among the Malayalis there is one instance (subject No 43) of a pelidisi of 87 There is no question but that this woman was very much under-nourished Her body weight was very small but her height was nearly up to the average and her sitting height a little higher than the average This is the only subject in the entire series of Indian women who has a pelidisi under 90 There are, however, among this group of Malayalis two subjects with pelidisi of 93 and one subject with a pelidisi of 92 None of the Telugus, the Coorgs, or the Kanarese would, from the pelidisi alone, be judged as having a low state of nutrition, with the possible exception of subject No 46 with a pelidisi of 93, and even here there would be only a moderate degree of under-nutrition if the pelidisi is applied as an index It is highly improbable, therefore, even when the shorter stem length is taken into account, that we are dealing here, except in one instance, with any cases of distinct under-nourishment

Physiological measurements on Indian women

Three physiological factors have been studied for all subjects, pulse rate, respiration rate, and especially the oxygen consumption The results of these measurements are recorded in Table II, grouped by race and increasing age, as in Table I In Table II the results are all expressed in values per minute, and each value in the table represents the average for a given day, based upon two, usually three, or not infrequently four(1) well-agreeing periods of measurement for the day With all except one subject (No 34) observations were made on at least two days, usually within a few days of each other and always inside of a month Hence we have recorded in the date column only the month and year of the measurements In some instances, as for example, with subject No 3, experiments were made on four separate days, and with subject No 11 on six days

Mouth temperature—In accordance with the custom of the Nutrition Laboratory, the mouth temperature of each subject was taken just prior to each experiment, with the object of ruling out any distinctly febrile condition In the majority of cases the mouth temperature was well within normal limits, showing no tendency to be predominantly low or high In the only two instances where febrile temperatures were found the metabolism measurements have not been used

Pulse rate—The grand average of the pulse rates of the Tamils is 69 beats per minute, of the Malayalis 64 beats, and of the fewer Telugus 72 beats The average for all subjects, including the Coorgs and Kanarese, is 68 beats In the series of values shown in Table II the second pulse rate is on a later day

(1) Du Bois (*Journal of Nutrition*, 1930, 3, p 220) rightly objects to the use of a single minimum value

than the first. It may have been determined two, four, or even more days later. The pulse rate on the second day is higher almost as often as it is lower. Specifically high pulse rates, that is, 80 or above, are noted with subjects Nos. 3, 27, 41, 50, and 52. The only very low pulse rate is that for subject No. 30, a Malayali with a rate of 48 beats on two days. Ideally a comparison of the pulse rates of these Indian women with the pulse rates of western women should be made on the same age basis. The average pulse rate of 90 American women (average age 30 years) studied by Harris and Benedict(1) is 69 beats per minute. This is essentially the same as the grand average for all of our Indian women. Hence the pulse rates of these Indian women are not significantly different from those of western women.

Respiration rate—The respiration rate, as studied with the type of respiration apparatus employed in this research, is always subject to a possible influence of the mouthpiece breathing-appliance. The experience of the Nutrition Laboratory has been that usually the introduction of the mouthpiece has a tendency automatically to lower somewhat the respiration rate. The grand averages of the respiration rates for the three main groups of Indian women are 19, 20, and 17 for the Tamils, the Malayalis, and the Telugus, respectively. An inspection of the data, however, shows widely different values. Thus, the highest rate is 34 respirations per minute with subject No. 40 and the lowest is 10 with subject No. 30. There is altogether too little recorded evidence on the respiration rate of western women measured when the subject was breathing through a mouthpiece, to enable a comparison with these Indian women. But the fact that 21 western women whom we have measured in Madras under the same condition as the Indian subjects show an average rate of 14 respirations per minute with a maximum of 19 and a minimum of 8 is strongly suggestive of a significant difference. With the majority of the Indian women the respirations were very shallow. Figure 1 shows a typical record for a Tamil, where the rise and fall of the spirometer with each respiration is about 10 mm, representing a tidal air of about 213 cc. It seems probable that the more rapid rate with the Indians is in the nature of a compensation for the small amount of air taken in with each breath. In general the respirations of the western women measured in Madras are appreciably deeper than those of South Indian women.

Oxygen consumption—From the standpoint of studying racial metabolism the most important factor was the measurement of the oxygen consumption, which is taken as the direct index of the basal metabolism or the level of vital activity. The oxygen consumption was measured with every care, and the values on the different days usually agree well with each other. Subject No. 11 is a striking exception, however, for her oxygen consumption varies

(1) HARRIS, J. A., and BENEDICT, F. G., *Carnegie Inst. Wash. Pub. No. 279*, 1919, Table XV, p. 66.

TABLE II
Pulse rate, respiration rate, and oxygen consumption of Indian women under basal conditions
(Values per minute)

Subject number	Date	Pulse rate	Respiration rate	Oxygen consumed c.c.	Subject number	Date	Pulse rate	Respiration rate	Oxygen consumed c.c.
Tamils					Malayalis				
1	Nov 1928	78, 68	24, 26	151, 149	28	Dec 1928	52, 53	22, 17	155, 155
2	Feb 1929	71, 67	17, 16	154, 149	29	Nov 1929	54, 55	24, 25	151, 149
3	Jan 1929	80, 81	18, 16	153, 165	"	"	54	23	143
"	"	74, 78	15, 16	153, 155	30	Nov 1929	48, 48	11, 10	163, 160
4	Jan 1929	71, 66	17, 13	166, 161	31	Oct 1928	67, 73	20, 19	167, 173
"	"	65	14	156	"	Aug 1929	68	20	171
5	Aug 1929	69, 64	29, 28	149, 146	32	Dec 1928	56, 61	21, 22	138, 136
6	Nov 1928	69, 69	21, 22	143, 148	"	Oct 1929	57	20	137
7	Mar 1929	66, 67	24, 20	140, 143	33	Oct 1929	60, 59	24, 24	160, 155
8	Oct 1928	68, 66	14, 16	151, 149	34	Aug 1928	67	18	164
9	Dec 1928	72, 62	20, 21	152, 178	35	Sept 1928	73, 66	21, 19	160, 156
"	"	63, 63	19, 17	159, 142	36	Aug 1929	60, 64	19, 20	121, 120
10	Nov 1928	76, 65	20, 20	143, 143	37	Oct 1928	69, 70	22, 22	148, 145
11	Sept 1928	75, 74	27, 27	146, 146	38	Nov 1929	50, 48	17, 17	158, 149
"	"	63, 69	27, 25	162, 148	39	Oct 1928	67, 70	23, 22	159, 156
"	Nov	77, 76	19, 22	173, 174	40	Nov 1928	72, 74	30, 33	152, 158
12	Aug 1929	58, 57	22, 21	150, 145	"	"	70	34	151

13	Aug 1928	70, 73	15, 18	131, 138	41	Feb 1928	81, 78	17, 16	160, 152
"	Mar 1929	70, 74	13, 16	132, 137	42	Oct 1928	65, 61	17, 13	145, 144
14	Aug 1928	55, 59	20, 19	134, 126	43	Aug 1928	77, 77	13, 16	127, 131
"	Aug and Sept 1929	52, 56	19, 16	129, 129	44	Feb and Mar 1930	73, 78	15, 16	157, 159
15	July 1929	65, 62	24, 22	159, 154	Average		64	20	151
16	Feb 1930	63, 67	16, 16	133, 127	Telugus				
17	Feb 1930	70, 69	15, 15	155, 149	45	Oct 1928	74, 74	21, 23	149, 154
18	Jan 1930	73, 72	19, 17	164, 156	46	Feb 1929	77, 77	18, 14	145, 140
19	Aug 1929	69, 70	17, 16	134, 135	47	Oct 1928	69, 67	18, 17	155, 154
20	July 1930	79, 77	24, 24	184, 180	48	Nov 1928	64, 61	18, 17	147, 146
21	Feb 1930	68, 66	17, 15	165, 167	49	Sept 1928	73, 72	16, 13	153, 149
22	Oct 1928	63, 57	33, 23	148, 154	50	Feb 1930	75, 75	12, 18	148, 139
23	Dec 1929	69, 65	20, 20	140, 143	"	Mar 1930	82	14	145
24	Oct 1928	70, 67	14, 13	138, 137	Average		72	17	148
"	July 1930	71, 71	14, 15	142, 139	Coorgs				
25	Mar 1929	61, 69	14, 14	145, 144	51	Dec 1928	81, 81	24, 23	144, 156
26	Feb 1930	70, 74	19, 15	173, 185	"	"	72	20	154
27	Oct 1928	83, 73	16, 18	161, 155	52	Sept 1928	69, 68	23, 21	155, 158
Average		69	19	150	Kanarese				
					53	Dec 1928	73, 71	21, 20	148, 154
					54	Feb 1930	66, 62	18, 17	151, 150

considerably The average oxygen consumption of the three largest groups of Indian women is 150, 151 and 148 c c per minute respectively The range in the values for the different subjects is considerable Thus, the minimum value among the Tamils is 129 c c with subject No 14 The maximum value is 185 c c with subject No 26 Among the Malayalis the minimum value is 120 c c with subject No 36 and the maximum value is 173 c c with subject No 31 It is interesting to note that subject No 30, who has the lowest pulse rate and the lowest respiration rate, has an oxygen consumption considerably higher than the average for this group Among the Telugus the grand average is 148 c c, the minimum is 140 c c with subject No 46, and the maximum is 155 c c with subject No 47 The few data for the Coorg and the Kanares show essentially the same general picture noted with the three main groups

One of the best indices as to whether the measured metabolism may be considered basal and not unduly affected by the physical state of the subjects or their unfamiliarity with the apparatus is the agreement of results on two nearly consecutive days On examining the figures in Table II we find that frequently the lower value occurs on the second day and yet by no means consistently enough to indicate that it is a regular rule, for frequently the higher value appears on the second day In general the values are sufficiently near each other for us to believe that the experiments were made without an undue amount of apprehension or difficulty on the part of the subjects and that we have a true index of the basal metabolism of these individuals existing at the time of measurement The third and fourth series of measurements on Nos 13, 14, 31, and 32, made at an interval of several months after the earlier measurements and checking them remarkably closely, furnish further evidence that our results probably represent as close an approximation to the true basal metabolism of these women as has been determined with any series of women studied under like conditions for the same length of time

The average oxygen consumption per minute of these Indian women is not far from 150 c c The average oxygen consumption of 103 American women studied by HARRIS and BENEDICT(1) was 194 c c per minute But the American women were on the average nearly 10 or 11 kg heavier and about 8 cm taller than our Indian subjects, and it is not justifiable to make a direct comparison of the oxygen consumption of these two races without a critical analysis of the relation of this factor to body size

Total heat production—The basal heat production was computed in all cases from the oxygen measurement per minute, as shown in Table III, using for each woman an average of all the oxygen measurements obtained with her on different days This means that in the majority of instances the values for two days were averaged In others values for as many as four days, and

(1) HARRIS, J A, and BENEDICT, F G, *Carnegie Inst Wash Pub* No 279, 1919, Table D, pp 44-47

TABLE III

Comparison of average basal metabolism of Indian women with standards of Harris and Benedict, and Aub and Du Bois

Subject number	NUMBER OF EXPERIMENTAL		O ₂ per minute c c	HEAT PRODUCTION PER 24 HOURS		DEVIATION FROM STANDARD OF	
	Days	Periods		Total cal	Pcr sq m cal	Nutrition Laboratory per cent	Aub Du Bois per cent
Tamils							
1	2	6	150	1,042	772	-16.6	-19.6
2	2	8	152	1,056	792	-15.8	-17.6
3	4	11	157	1,091	794	-13.0	-17.3
4	3	7	161	1,119	805	-10.5	-16.2
5	2	7	148	1,028	737	-18.1	-19.2
6	2	6	146	1,014	728	-19.2	-20.3
7	2	6	142	987	745	-21.4	-18.4
8	2	6	150	1,042	729	-16.7	-20.1
9	4	9	158	1,098	850	-12.3	-6.8
10	2	6	143	994	736	-20.5	-19.3
11	6	15	158	1,098	769	-12.1	-15.8
12	2	6	148	1,028	748	-18.0	-18.0
13	4	12	135	938	728	-25.2	-19.2
14	4	10	130	903	640	-29.8	-27.9
15	2	6	157	1,091	750	-17.3	-15.6
16	2	6	130	903	684	-26.5	-23.0
17	2	6	152	1,056	794	-15.0	-10.6
18	2	6	160	1,112	842	-9.8	-5.2
19	2	6	135	938	730	-21.9	-17.8
20	2	6	182	1,265	748	-13.0	-15.8
21	2	6	166	1,153	721	-17.5	-18.9
22	2	4	151	1,049	772	-16.0	-13.2
23	2	6	142	987	723	-20.2	-18.6
24	4	12	139	966	721	-19.7	-18.8
25	2	6	145	1,007	750	-17.8	-15.6
26	2	6	179	1,244	745	-14.2	-15.0
27	2	6	158	1,098	784	-10.6	-10.5
Average			150	1,048	753	-17.4	-16.8

TABLE III—*contd*

Subject number	NUMBER OF EXPERIMENTAL		O ₂ per minute c c	HEAT PRODUCTION PER 24 HOURS		DEVIATION FROM STANDARD OF	
	Days	Periods		Total cal	Per sq m cal	Nutrition Laboratory per cent	Aub Du Bois per cent
Malayalis							
28	2	4	155	1,077	718	-13.8	-25.2
29	3	6	148	1,028	693	-17.9	-27.8
30	2	6	162	1,126	774	-10.2	-19.4
31	3	8	170	1,181	870	-5.3	-4.6
32	3	8	137	952	661	-23.8	-28.7
33	2	6	158	1,098	777	-12.4	-14.9
34	1	3	164	1,139	764	-8.9	-14.0
35	2	6	158	1,098	814	-12.2	-8.4
36	2	7	121	841	650	-33.0	-26.9
37	2	4	147	1,021	727	-18.6	-20.4
38	2	6	154	1,070	706	-14.7	-20.5
39	2	6	158	1,098	766	-15.2	-13.8
40	3	9	154	1,070	762	-16.5	-14.1
41	2	6	156	1,084	704	-17.8	-20.8
42	2	6	145	1,007	674	-23.2	-24.1
43	2	6	129	896	741	-21.1	-16.6
44	2	6	158	1,098	814	-9.2	-8.4
Average			151	1,052	742	-16.1	-18.2
Telugus							
45	2	4	152	1,056	810	-15.8	-11.3
46	2	6	143	994	739	-19.5	-16.8
47	2	6	155	1,077	746	-16.4	-16.1
48	2	6	147	1,021	717	-20.6	-19.3
49	2	5	151	1,049	766	-15.8	-13.8
50	3	10	144	1,001	735	-18.4	-17.2
Average			148	1,033	752	-17.8	-15.8

TABLE III—concl'd

Subject number	NUMBER OF EXPERIMENTAL		O ₂ per minute cc	HEAT PRODUCTION PER 24 HOURS		DEVIATION FROM STANDARD OF	
	Days	Periods		Total cal	Per sq m cal	Nutrition Laboratory per cent	Aub-Du Bois per cent
Coorgs							
51	3	7	151	1,051	735	-15.9	-19.4
52	2	3	157	1,088	783	-13.9	-11.9
Kanarese							
53	2	6	151	1,049	783	-16.1	-18.5
54	2	6	151	1,046	712	-19.4	-19.9

in one case six days. An average respiratory quotient of 0.82 was assumed(1), and the total heat production per 24 hours was determined directly from the average oxygen consumption per minute, according to calculations published by Carpenter(2). On the average, the calculated total 24-hour basal heat production of the three main groups of subjects is 1,048, 1,052 and 1,033 calories, respectively, and the grand average is 1,050 calories.

Heat production per square meter of body surface—A standard of comparison commonly accepted by physiologists is that of the heat production per square meter of surface area per 24 hours. Since comparison of the heat production per unit of surface area shows essentially the same picture as do comparisons upon other bases, this standard may be as well used here as any other. We have accordingly divided the total heat production of each subject by her surface area. These areas were not measured but, with the use of the height and weight measurements, were computed from the Du Bois chart(3), which has been shown to give satisfactory results. The heat production per square meter of body surface per 24 hours is distinctly low, averaging 753 calories for the Tamils, 742 calories for the Malayalis, and 752 calories for the few Telugus. The heat production of 103 western women studied by

(1) In this connection it is interesting to note that MUKHERJEE and GUPTA (1931), *Ind Jour Med Res*, **18**, 3, p. 807, found the average respiratory quotient of 18 Bengali men to be 0.84. The diet of the Bengalis is essentially similar to that of the Madrasis.

(2) CARPENTER, T. M., *Carnegie Inst Wash Pub* No. 303A, 1924, Table XIV, p. 105.

(3) DU BOIS, D., and DU BOIS, E. F., *Arch Intern Med*, 1916, **17**, p. 865. It has been shown by WADDELL, S. S., HAN, C. H., and CH'EN, Y. P. (*Chinese Jour Physiol*, 1928, Report Series No. 1, p. 25), that the error in the application of the Du Bois height-weight formula to the estimation of the surface area of Chinese adults is little, if any, greater than with American subjects. It may be assumed, therefore, until proved to the contrary, that the Du Bois height-weight formula and chart may be used with justification in the case of Indian women.

HARRIS and BENEDICT averaged 850 calories per square meter of body surface per 24 hours

Comparison of basal metabolism of Indian women with western prediction standards—Although the average heat production of women of the American series is 850 calories per square meter of surface area per 24 hours, no one standard can be applied to all women. Hence we have compared the basal metabolism of our individual subjects with two commonly accepted standards for western women, one the Harris-Benedict predictions for women(1) and the other the Aub and Du Bois predictions(2). Since the Harris-Benedict predictions are applicable in the case of women only for ages of 21 years and above, for ages below 21 years (and many of our subjects were under 21 years) we have accepted the prediction of 1,250 calories as the probable total heat production per 24 hours. This average value is based upon measurements made by the Nutrition Laboratory upon groups of Girl Scouts, ranging in age from 12 to 18 years, with whom it was found that the total 24-hour basal heat production in all cases was close to 1,250 calories, irrespective of age, weight, or height(3). Since this average value of 1,250 calories was obtained in experiments when the Girl Scouts were asleep at night, it is a low rather than a high standard. The Aub and Du Bois predictions for women, which are applicable to girls as young as 14 years, are based upon a deduction of 7 per cent from their standards for males. Since the Aub and Du Bois predictions were drawn in very large part from the Nutrition Laboratory series of basal measurements on humans, it is not surprising that their series of predictions are essentially the same as the Harris-Benedict series.

The percentage deviations of the actually measured basal heat production of our Indian subjects from the standards of the Nutrition Laboratory and those of Aub and Du Bois are given in Table III. *In all cases the values are minus.* The largest deviation from the Nutrition Laboratory prediction among the Tamils is with subject No. 14, -29.8 per cent. There are seven values of

(1) HARRIS, J. A., and BENEDICT, F. G., *Carnegie Inst. Wash. Pub. No. 279*, 1919, Tables III and IV, pp. 260, *et seq.*, reprinted by CARPENTER, T. M., *Carnegie Inst. Wash. Pub. No. 303A*, 1924, Tables XXVI and XXVII, pp. 116, *et seq.*, by GRAFE, E., *Die Pathol. Physiol. d. Gesamtstoff- u. Kraftwechsels bei der Ernährung d. Menschen*, Munich, 1923, Tables I to IV, pp. 488-499, by BENEDICT, F. G., *Abderhalden's Handb. d. biolog. Arbeitsmethoden*, 1924, Abt. IV, Teil 10, Tables XXI to XXIV, pp. 657-674. Predictions based upon the HARRIS-BENEDICT data are also given by KESTNER, O., and KNIPPING, H. W., *Die Ernährung d. Menschen*, 2nd ed., Berlin, 1926, pp. 5, *et seq.*, KNIPPING, H. W., and RONA, P., *Praktikum der physiol. Chemie*, III, *Stoffwechsel u. Energiewechsel*, Berlin, 1928, pp. 56, *et seq.*

(2) AUB, J. C., and DU BOIS, E. F., *Arch. Intern. Med.*, 1917, **19**, p. 831.

(3) BENEDICT, F. G., and HENDRY, M. F., *Boston Med. and Surg. Jour.*, 1921, **184**, pp. 217, 257, 282, 297, and 329, BENEDICT, F. G., HENDRY, M. F., and BAKER, M. L., *Proc. Nat. Acad. Sci.*, 1921, **7**, p. 10, BENEDICT, F. G., *Boston Med. and Surg. Jour.*, 1923, **188**, p. 127.

-20 per cent or more, that is, these seven women have a metabolism 20 per cent or more lower than the Nutrition Laboratory standards for young girls or the Harris and Benedict standards for women over 21 years. With the Aub and Du Bois predictions the values are somewhat different. The greatest deviation is with the same subject noted before (No 14) with -27.9 per cent and there are four subjects that are 20 per cent or more lower than the Aub and Du Bois prediction. On the average, the 27 Tamils have a basal metabolism 17.4 per cent below the Nutrition Laboratory standard and 16.8 per cent below the Aub and Du Bois standard. The whole picture therefore is of a very low metabolism. The agreement between the average deviations from the two standards is a striking demonstration of the fact that in general the two types of prediction run closely together. There are a few cases where there is a considerable difference, but with most of the Tamils the percentage deviations by the two standards agree within 2 or 3 per cent, or better.

Among the 17 Malayalis the greatest deviation from the Nutrition Laboratory predictions is with subject No 36, -33 per cent, and there are four subjects whose metabolism is more than 20 per cent below. The average deviation for the group is -16.1 per cent. By the Aub and Du Bois predictions the lowest metabolism is that of subject No 32, who shows a deviation of -28.7 per cent, and there are eight subjects whose metabolism is 20 per cent or more below this standard. The Telugus and subjects Nos 51 to 54 show much the same picture, for all of the deviations from both standards are minus and they average not far from 16 to 18 per cent.

In general the metabolism of all the groups on either basis of prediction averages from 16 to 18 per cent below the standards for normal western women, with a large number of subjects more than 20 per cent below the standards and with seven of fifty-four presumably healthy women 25 per cent or more below the western standards. All of this speaks for a very low metabolism with these Indian women. It must be emphasized again that we are not dealing with hospital subjects nor with 'hospital normals' or convalescents, but with presumably healthy, normal Indian women engaged in study or in teaching in a college.

Although the general picture is of a very low metabolism, it is important to note more specifically those cases that are 25 per cent or more below the western standards and to examine the characteristics of the individuals themselves. Thus, among the Tamils the first subject with this low metabolism is subject No 13. Reference to Table I shows that No 13 is 20 years of age, with a low body weight, 38.1 kg, nearly the lowest of the series, with a height a little less than the average, and with a pelvis of 97, which is normal. In other words, there is nothing particularly significant in the general physical characteristics of this girl to explain her very low metabolism. The next in the series is No 14 with a metabolism 29.8 per cent below the Nutrition Laboratory standard and 27.9 per cent below the Aub and Du Bois standard. This subject has a body-weight a little above the average for her group, a

height only 1 cm below the average, and a pelvis of 101. Here again, no particular reason for this exceedingly low metabolism can be derived from the configuration or the general physical make-up of this girl. The next case in the series is No. 16 with deviations of -26.5 and -23.0 per cent. The physical characteristics of this girl again show nothing abnormal. The body-weight is a little low, but there is a correspondingly low height and a pelvis of 99. Among the Malayalis, subjects Nos. 28 and 29 both show deviations from the Aub and Du Bois predictions greater than 25 per cent. Both of these girls weighed more than the average for their group and were a little taller than the average. Their pelvis were 99 and 98, respectively. Subject No. 32 has a value of -28.7 per cent by the Aub and Du Bois predictions. This subject has a body weight of 45.4 kg and a height of 159 cm, with a pelvis of 99, or the average build for this group. Subject No. 36 has an extraordinarily low metabolism, 33 per cent below the Nutrition Laboratory prediction standard and 26.9 per cent below the Aub and Du Bois standard. She has a low body-weight, indeed the next to the lowest in the series of Malayalis. Her height is nearly the same as the average, but her pelvis is 92, the first indication of a low state of nutrition. An explanation in part for the low metabolism of subject No. 36 therefore might be that she was distinctly under-nourished. An analysis of the physical characteristics of those subjects having deviations of between -20 and -25 per cent shows essentially the same picture as that with the subjects 25 per cent below the standards.

GENERAL CONSIDERATIONS ON THE LOW METABOLISM OF THE INDIAN WOMEN

The low values noted with these Indian women are not readily explainable by differences in configuration or evidences of under-nutrition, but they are so low as to lead one to conjecture as to what are the probable causes. The first cause that suggests itself is that we have to deal here with a real racial difference, which seems to be strikingly indicated in the case of the Chinese and Japanese women studied in North American colleges. It is believed now that the Aub and Du Bois predictions and the Nutrition Laboratory predictions are probably a little too high for women and that they should be lowered approximately 5 per cent (1). If this correction is applied, the basal metabolism of these Indian women would be on the average from 11 to 13 per cent below the western standards, with a considerable number of subjects still showing values 20 per cent or more below, even after the correction is applied.

Of the possible factors affecting metabolism there are, outside of the endogenous factor of racial metabolism, other endogenous factors, among them a low protein metabolism due either to low protein intake or to low protein

(1) MacLEOD, G., CROFTS, E. E., and BENFICT, F. G., *Amer Jour Physiol*, 1925, **73**, p. 462, *ibid*, *Proc Nat Acad Sci*, 1925, **11**, p. 343, BENFICT, F. G., *Amer Jour Physiol*, 1928, **85**, p. 619

absorption (1) This factor of the protein metabolism is being studied by us. The reaction to ingested protein, or the specific dynamic action of protein should be studied, to determine whether individuals with such a low metabolism show any different reaction from that of individuals with a higher metabolism. Exogenous factors are climate, temperature, and humidity. Records concerning these are being secured in all of our experiments and will be analysed subsequently. Finally, there is the possibility that Orientals as a whole have a greater degree of relaxation and repose during rest than have the westerners. It is conceivable that the Orientals can assume during repose a position of complete relaxation whereas with the westerners there may be some nervous tension, even though slight. It is our belief that this factor of relaxation could best be studied with the various races during normal sleep. It has been found that with westerners sleep lowers the metabolism approximately 10 per cent. The question arises: Will normal sleep materially lower the basal metabolism of these Oriental women, who already have such a low metabolism? Studies of the metabolism during sleep are in progress.

The results reported here are of value alone as establishing standards for Indian women for use in physiological laboratories and hospitals in India. We believe that there is definite evidence of a racial factor. That this factor will explain entirely the low metabolism we are not at all sure. When the factors of protein ingestion and absorption, degree of muscular repose, and climate are more thoroughly studied, we shall be in a much better position to secure a complete explanation of this extraordinarily low metabolism.

SUMMARY

The basal metabolism of 54 women (Tamils, Malayalis, Telugus, Coorgs, and Kanarese) in South India, of ages ranging from 17 to 31 years, has been measured with the Benedict portable apparatus, supplemented by graphic records. The average wet-bulb room temperature was 25°C, dry bulb 27°C, with slight variations from the mean through the year.

No significant differences between the various groups of Indians appear in any of the measurements made. The body weights of the subjects are low compared with standards for westerners. The average weight is 45 kg, with a range of from 33.4 to 67.6 kg. The standing heights are also low by western standards, averaging 154 cm and ranging from 145 to 167 cm. Actually measured sitting heights average about 4 cm less than the calculated sitting heights (one-half the standing height plus 5 cm) and indicate that these subjects have relatively shorter stem lengths and longer legs than westerners.

The state of nutrition, as indicated by the pelidisi, is normal and compares favourably with the pelidisi of normal western women.

(1) For a discussion of this latter factor in the problem of nutrition in India see McCay, D., *The protein element in nutrition*, *Internat Med Monographs*, London and New York, 1912.

The pulse rates are not significantly different from those of western women, ranging from 48 to 83 beats per minute and averaging 68 beats. Although the difficulty of getting normal respiration rates with mouthpiece breathing appliances is recognized, the findings strongly suggest that the respiration rate is higher in Indian women than in western women living in India. This higher rate, averaging 19 respirations per minute, is coincident with very shallow breathing.

The oxygen consumption per minute averages 150 c c and varies from 120 to 185 c c. The average percentage deviation of the basal heat production from the Harris-Benedict standards is -16.9 per cent with variations of from -5.3 to -33.0 per cent. The average deviation from the Aub and Du Bois standards is -17.2 per cent, varying from -4.6 to -28.7 per cent.

Probable factors to explain this very low metabolism of South Indian women are suggested. There is evidence that there is a definite racial factor. Other possible causes are (1) a low protein metabolism, (2) tropical conditions of climate, and (3) a state of relaxation during repose as complete as that which is found during sleep among westerners. Further work is in progress along these three lines.

PHLEBOTOMUS STANTONI NEWSTEAD, 1914 AND SOME OTHER SIAMESE SANDFLIES

BY

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[Received for publication, January 22, 1931]

A NUMBER of specimens of *Phlebotomus* were collected during a visit to Siam in December 1930. As there seems to be no previous mention of the occurrence of *Phlebotomus* in this country, these seem worthy of record, more especially as two females of *P. stantoni* were obtained.

In Bangkok sandflies seemed very rare at this time of the year and only 5 specimens were captured (*P. stantoni*, 2 ♀♀, *P. barlyi* var. *campester*, 2 ♀♀, 1 ♂). On a train journey from Bangkok to Chiangmai in Northern Siam, numerous sandflies were collected near the electric lights in the train and these all proved to be *P. squamipleuris* (7 ♀♀, 33 ♂♂).

Phlebotomus stantoni was originally described by Newstead (1914) from one female specimen collected at Kuala Lumpur, Federated Malay States (15 vi 14). Since that time no other specimens seem to have been recorded. The original description of this specimen was brief and left considerable uncertainty as to whether it was identical with *P. argentipes* Ann and Brun, 1908 (Sinton, 1923, 1928). An examination of the two Siamese specimens leaves no doubt that the two species are distinct. A more complete description of *P. stantoni* (♀) is therefore given below. The male is unknown.

Phlebotomus stantoni NEWSTEAD, 1914

The original description given by Newstead (1914) was as follows —

♂ —Length, 21 mm. Wing, 19 mm.

♀ —Unknown.

A medium-sized species distinguishable by the silvery grey, recumbent hairs on the venter of the abdomen, in the palpi, by the unusually short 4th segment, and the relatively short terminal segment, by the vertical of long hair-like scales on the 2nd segment of the antennæ, and also by the scales on the tarsi being arranged in broad distinct bands,

Abdominal hairs more or less erect *dorsally*, and arranged in indefinite tufts, *those of the venter recumbent* and silvery grey, standing out in marked contrast to those on the dorsum, which are faintly infuscated. Legs tarsi clothed with dull silvery scales arranged in complete and well defined bands or zones, when mounted in balsam and examined in optical section the arrangement of the scales is seen to be strikingly characteristic, but it is curious to note that the integument in the inter-zonal spaces is covered with cicatrices, though there is no trace of either scales or hairs arising from any of them. Antennæ with geniculated spines on all the segments of the flagellum, with the exception of the terminal one, i.e., 3rd-15th inclusive, these for the most part at least are of great length, the tips reaching nearly to the bases of the spines on the succeeding segment, 2nd segment (scape) with a single verticil of long stout hair-like scales, 3rd segment long, the tip reaching almost to the tip of the proboscis, hairs relatively long and stout, and the smaller segments of the flagella rather densely clothed with them, sensoria on the terminal segments with a few rather conspicuous hairs. Palpi rather short and slender, 3rd and 5th segments the longest and about equal in length, 4th unusually short, being a little more than half the length of the 2nd, formula, 1, 4, 2 (3, 5). Wings with the 1st longitudinal vein terminating well in advance of the anterior branch of the 2nd, anterior branch of the 2nd longitudinal vein one and a half times as long as the distance between the two forks, no further particulars can be given, as the margins of both wings are crumpled.

FEDERATED MALAY STATES Kuala Lumpur, 1 ♀ (type), 15 vi 14 (Dr A T Stanton)

PHLEBOTOMUS STANTONI (♀)

The two specimens here described were only discovered after very prolonged and careful daily searches. One specimen was captured in a bedroom and the other in a bathroom in the Phya Thai Hotel, Bangkok, Siam, between 5th and 12th December, 1930. Although the electric lights were burning brightly, the insects did not seem to be attracted to their immediate vicinity. One other female specimen was seen but no males.

Through the kindness of Professor R. Newstead, FRS, I had an opportunity in 1926 of seeing the type specimen of this species. Unfortunately the specimen had been crushed since the original description was made, but some rough measurements were made by me and these are given in Table I for comparison with the Siamese specimens.

Appearance of dry specimens from Siam

The species is a medium-sized one, belonging to the erect-haired group. Its general appearance was dark brown, but the sides of the thorax looked a yellowish orange colour. The eyes were black. The abdominal hairs were abundant. All those on the dorsum were markedly erect and inclined to be tufted. The ventral hairs were very recumbent. The colour of the dorsal hairs was very dark brown, while those on the venter were slightly lighter in colour, as were also those on the dorsum of the thorax. The integument of the abdomen and the dorsum of the thorax was dark brown, almost black, while the sides of the thorax, as well as the coxæ and trochanters, were honey-coloured. The wings showed a bluish-golden iridescence and were covered with greyish brown hairs. The halteres had black tips. The legs looked very

dark grey in some lights but by reflected light were silvery The scales on the tarsi showed a marked banding The antennæ and palps were grey

Appearance in stained and mounted specimens

The measurements and ratios of the type and the two Siamese specimens are shown in Table I

The average total length of the insect was 2.5 mm (2.4–2.67 mm)

The buccal cavity (Plate II, fig 5) shows a row of two large teeth and several small ones in its anterior part, which is different from the row of 4 to 6 equal medium-sized teeth seen in *P argentipes* (cf Christophers, Shott and Barraud, 1926, Plate XXII, fig 30) No pigmented area is present

The pharynx (Plate II, fig 4) is markedly dilated posteriorly Its length is about 2.3 times its greatest breadth, while the width of the narrow anterior portion is about 2.4 times this breadth The armature in its posterior portion consists of a series of low transverse ridges with minute spines on their free edges Anterior to these the ridges become more conspicuous but do not carry spines In the middle line anteriorly the armature consists of a number of pointed teeth which blend with the ridges laterally The armature resembles that of *P argentipes* (♀) but the median teeth are more numerous, longer and more slender in *P stantom* (cf Sinton and Barraud, 1928, Plate XXIX, fig 5)

The antennæ (Plate II, figs 10, 11 and 12) have a total length of more than 4 times that of segment III and nearly 6 times that of segments XII to XVI Segment III is long, being greater than that in *P argentipes* Its distal end does not extend quite so far as the tip of the proboscis The combined length of segments III and IV almost equals that of segments XII to XVI The antennal formula is 2 over III to XV The geniculated spines are very long and extend beyond the inter-segmental articulations, while in *P argentipes* they do not do so (cf Sinton, 1925, Plate LIV, figs 3 and 4)

The palps (Plate II, fig 2) have a formula of 1, 2, 4, 3, 5 and the relative lengths of the segments are very similar to those in *P argentipes* Segment 4 is very short, while segment 3 is approximately equal to the combined length of segments 1 and 2 Newstead's spines are situated on the middle third of segment 3 and number about 15 on each palp

The wing (Plate II, fig 1) is about 3.3 times as long as broad There is some variation between the ratios of the different parts of the wings on opposite sides of the same specimen δ is comparatively short The proximal fork of the 2nd longitudinal vein is much nearer the base of the wing than the fork of the 4th and the origin of the 3rd vein is closer to the termination of the subcostal vein than in *P argentipes* (cf Sinton, 1925, Plate LIV, fig 1)

The hind leg is relatively long

The female genitalia (Plate II, figs 3, 7, 8 and 9) The spermatheca has a crenulated outline as in other members of the erect-haired group This structure (Plate II, fig 3) is torpedo-shaped with a tendency to a short neck

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The crenulations number about 14 to 16 and the head is of medium size. The *spermathecal ducts* (Plate II, fig 7) quickly unite to form a very long common duct, which is nearly 4 times as long as that seen in *P argentipes* (Plate II, fig 6). The *post-genital ridge* (Plate II, fig 8) carries two spines. The *furca* (Plate II, fig 9) shows a few ill-marked serrations at its base and has not the markedly inverted inner margins seen in *P argentipes* (cf Sinton, 1927a, Plate VII, fig 20).

Identity of the Siamese specimens

From the original description given by Newstead (1914) and the very close similarity between the measurements, ratios, etc., of the type and the Siamese insects shown in Table I, there is no doubt that the latter specimens are *P stantoni*. Some rough drawings made from the type specimen support this identity.

Differential diagnosis of P stantoni (♀)

The species belongs to the erect-haired group and the morphology of the spermatheca is distinctly different from that described in any of the other members of this group.

The possibility that *P stantoni* might be the same species as *P argentipes* was suggested by Sinton (1923, 1928). In their general appearance the two species resemble each other. Both species have also somewhat similar wing venation, palpal measurements and spermathecae. Very distinct differences however exist as have been tabulated below —

Structure	<i>P argentipes</i> (♀)	<i>P stantoni</i> (♀)
Wing	Proximal fork of 2nd vein about level with fork of 4th	Fork of 2nd much nearer base of wing
	Base of 3rd vein very distant from end of subcostal	Not so distant
	α over β averages about 1.9	About 1.5
Antenna	Distal end of 3rd segment does not nearly reach tip of proboscis	Nearly reaches tip
	Geniculated spines short and not surpassing inter segmental junctions	Very long and surpassing junctions
Buccal cavity	With an anterior row of 4-6 teeth all of medium size and about equal length	With two large and several small teeth
Pharyngeal armature	With comparatively few stout median teeth anteriorly	With more numerous long, slender teeth
Female genitalia	Spermatheca carrot shaped	Torpedo shaped
	Short common spermathecal duct	Very long
	Furca with markedly inverted inner margins and usually conspicuous basal serrations	Not so

TABLE I

Phlebotomus stanton Newstead, 1914 (♀)

Structure		Length in mms of specimens number —			Remarks, average relative lengths, etc ‡
		1*	2†	3†	
Body	Clypeus and head	0 480	0 414	0 400	
	Thorax	0 650	0 700	0 628	
	Abdomen proper	1 070	1 414	1 400	
	Sup clasper	0 200	0 143	0 170	
	Total length	2 40	2 67	2 60	
Mouth	Labium	0 320	0 280	0 280	$\frac{P}{L} = 2.0$
	Pharynx, length		0 195	0 180	$= 2.3 \times \text{breadth}$
	Pharynx, breadth		0 084	0 081	$= 2.4 \times \text{narrowest width}$
Antenna	Segment III	0 287	0 285	0 280	$III > IV + V \quad IV = V = VI$ $IV + V + VI < XII - XVI \quad III + IV \approx XII - XVI$
	Segment IV	0 110	0 108	0 105	
	Segment V	0 110	0 108	0 110	Formula $\frac{2}{III - XV}$ $= 4.1 (4.25) \times XII - XVI, = 5.9 (5.9) \times III$
	Segment VI	0 110	0 110	0 110	
	Segments XII-XVI	0 400	0 417	0 400	
	Total length	1 700	1 700	1 643	
Palp	Segment 1	0 040	0 045	0 045	Formula—1, 2, 4, 3, 5 Rel lengths 6 2 14 8 20 6 10 28 0 $= 1+2$
	Segment 2	0 105	0 105	0 108	
	Segment 3	0 142	0 150	0 147	
	Segment 4	0 065	0 072	0 072	
	Segment 5	0 168	0 195	0 210	
	Total length	0 520	0 567	0 582	
Wing	Length	1 920	1 970	1 930	$= 3.2-3.3 (3.27) \times \text{breadth}, 0.54 \times \text{hind leg}$
	Breadth	0 570	0 600	0 600	
	α	0 450	0 437	0 428	$\frac{\alpha}{\beta} = 1.5-1.6 (1.6) \quad \frac{\beta}{\gamma} = 1.18 (1.12)$
	β	0 280	0 270	0 285	$\frac{\alpha}{\gamma} = 1.76-1.90 (1.80) \quad \frac{\delta}{\alpha} = 0.16-0.18$
	γ	0 250	0 228	0 243	(0.20)
	δ	0 090	0 080	0 071	$\frac{\alpha}{\epsilon} = 0.76 (0.76) \quad \frac{\alpha + \beta}{\theta} = 0.78 (0.77).$
	ϵ	0 590	0 570	0 570	
	θ	0 940	0 900	0 900	$\frac{\theta}{\epsilon} = 1.57-1.61 (1.59) \quad \frac{\text{Wing}}{\theta} = 2.16$
	π	0 100	0 128	0 128	(2.04)

TABLE I—concl'd

Structure		Length in mms. of specimens number —			Remarks, average relative lengths, etc ‡
		1*	2†	3‡	
Hind leg	Femur		0 757	0 785	
	Tibia		1 271	1 314	
	Tarsus, seg 1		0 785	0 843	
	Tarsus, segs 2-5		0 743	0 757	
	Total length		3 55	3 70	(Not including coxa and trochanter)
Gen	Spermatheca, length		0 057	0 060	= 3 6-3 8 × breadth
	Spermatheca, breadth		0 015	0 016	

* Rough measurements made from type specimen in 1926

† Measurements of two Siamese specimens

‡ The ratios in brackets are those of the type specimen for comparison

From the data given above it is evident that *P. stantoni* Newstead, 1914, is not synonymous with *P. argentipes* Ann and Brun, 1908, and must be included among the Asiatic species *

Phlebotomus bairlyi var *campester* SINTON, 1931

Two females and one male of this variety of *P. bairlyi* were collected at the same time as the specimens of *P. stantoni*, i.e., in a bedroom and a bathroom at the Phya Thai Hotel, Bangkok, Siam, between 5th and 12th December, 1930. This is the first record of this species outside the Indian Empire.

Phlebotomus squamipleuris Newstead, 1912

While on a railway journey from Bangkok to Chiangmai in Northern Siam, a number of *Phlebotomus* were captured in the vicinity of the electric lights in the train. This peculiar attraction of bright light for *P. squamipleuris* has already been pointed out (Sinton, 1927). All the specimens captured proved to be this species and were collected in the following places: (a) Between Paknampoh and Pistnuloke Railway Stations on 14th December, 1930 (5 ♀♀, 23 ♂♂) and (b) between Uttradit and Pistnuloke Railway Stations on 18th December, 1930 (2 ♀♀, 10 ♂♂). A solitary specimen of what seemed to be this species was observed near a light in a bungalow at Chiangmai on 17th December, 1930.

* Since writing the above, Dr C. Manalang has very kindly sent me some specimens of *P. philippinensis* Manalang, 1930. The spermathecae and pharyngeal armature of this species very closely resemble that of *P. stantoni*. The former species may be a variety of, if not identical with, *P. stantoni*. This synonymy can be settled when specimens of the male of *P. stantoni* are available.

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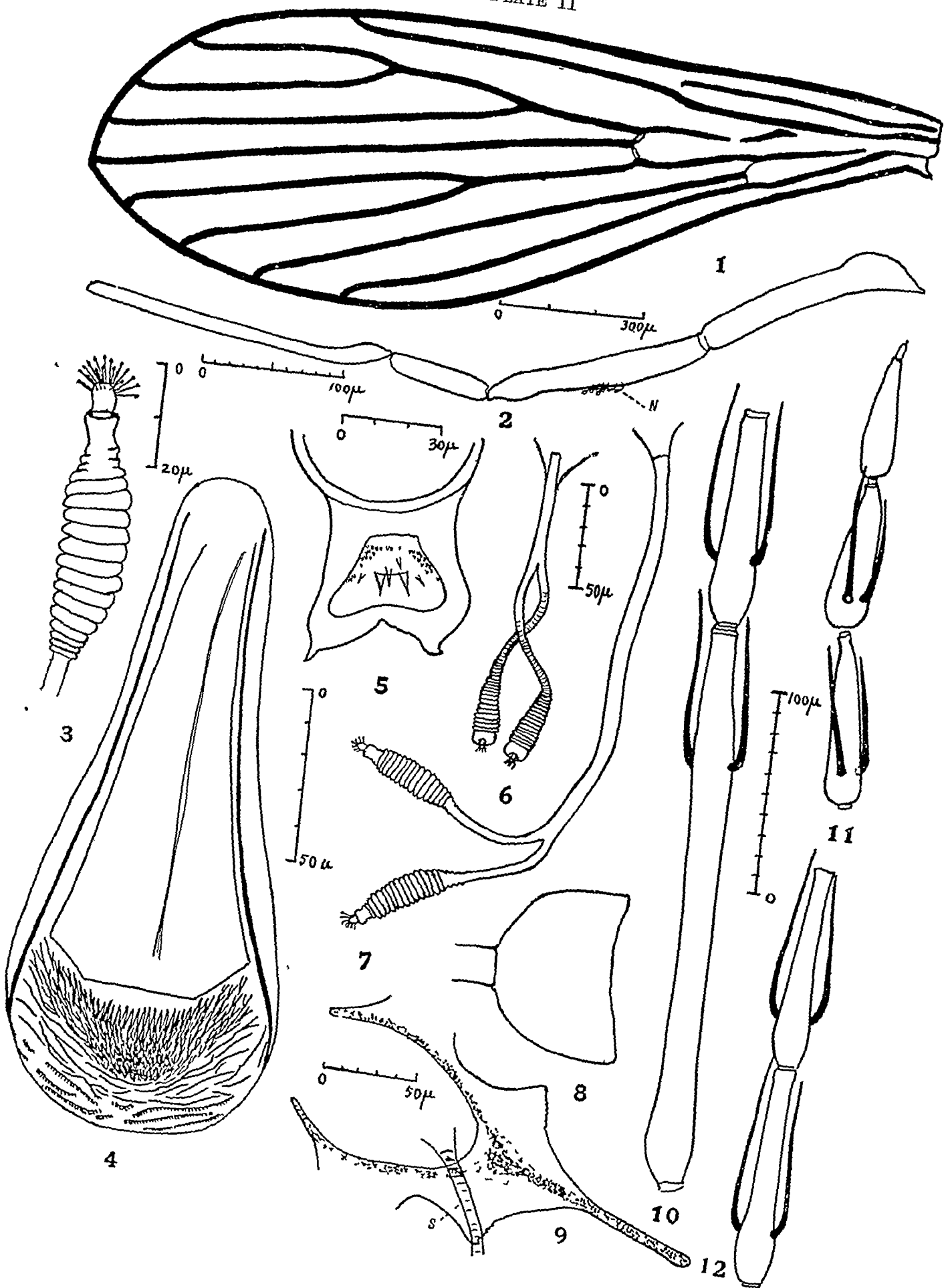
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EXPLANATION OF PLATE II

Phlebotomus stantoni (♀)

- Fig 1 Wing
,, 2 Palp N – Newstead's spines
,, 3 Spermatheca
,, 4 Pharynx
,, 5 Buccal cavity
,, 6 Spermathecae and ducts in *P argentipes*
,, 7 Spermathecae and ducts in *P stantoni*
,, 8 Post-genital plate
,, 9 Furca and termination of common spermathecal duct (S)
,, 10 Antennal segments III and IV
,, 11 Antennal segments XIV, XV and XVI
,, 12 Antennal segments VII and VIII

PLATE II



NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

Part XXIX.

PHLEBOTOMUS ARBORIS N SP

BY

MAJOR J A SINTON, M D, D SC, I M S

(Malana Survey of India, Kasauli)

[Received for publication, February 27, 1931]

A COLLECTION of about fifty *Phlebotomus* has kindly been given to me by Dr I M Puri. These specimens were obtained on 21st August, 1921, from cavities in large trees near the Marianbari Tea Estate in the Darjeeling District of the Bengal Terai. The majority of these specimens were *P zeylanicus* and *P pun* but there were about 20 males which appeared to be an entirely different species. Unfortunately no females of the species were captured. It is proposed that the name *P arboris* be given to this new species.

Phlebotomus arboris (♂)

The colour of the insects was a greyish or yellowish brown, and was distinctly lighter than the specimens of *P pun* with which they were found. The integument of the abdomen was dark brown, while the thorax had yellowish brown sides with a slightly darker dorsum. The abdominal hairs were abundant, markedly recumbent on the dorsum but ruffled on the venter. Their colour was yellowish brown. The wings had a golden blue iridescence and the hairs were a dark silvery grey. The scales on the legs were a similar colour but in some lights had a bluish effulgence. The hairs of the antenna projected markedly from the segments and did not lie close to them as in *P pun* (vide Sinton, 1931). The genitalia were very hairy and the intro-mittent organ was very dark brown, almost black.

Appearances in stained and mounted specimens

The measurements of the type and three co-type males are given in Table I, in which the averages, ratios, etc., of eight specimens are also shown

The *total length* was from 2.4 to 2.7 mm (av. 2.57)

The *buccal cavity* (Plate III, fig. 5) shows a row of small teeth which are inclined to form groups of 3 or 4. The pigmented area is distinct but not large. It is heart or goblet-shaped and in some instances is broken into several pieces.

The *pharynx* (Plate III, fig. 6) is not markedly dilated posteriorly and its greatest width is about 3 times its length. The armature consists of a series of slightly curved transverse ridges with short teeth along their free posterior margins.

The *antennae* (Plate III, figs. 2, 3, 4, 7 and 8) have single geniculate spines on all segments from III to XV, these spines are comparatively long and those on the terminal segments reach as far as the succeeding inter-segmental articulations. Some of them (Plate III, figs. 7 and 8) show a small basal projection similar to that described in *P. purni* (Sinton, 1931). The antenna is very long and is about 5 times the length of segment III, which is as long as the combined length of segments XII to XVI. The end of segment III passes much beyond the tip of the proboscis. The hair-like scales on the flagellum project more markedly than in *P. purni*.

The *palp* (Plate III, fig. 13) has a formula of 1, 2, 3, 4, 5 and the relative lengths of the segments averaged in eight specimens 2.2, 6.3, 9.0, 10, 19.7. Newstead's spines are situated on the basal fourth of the 3rd segment and are about 8 in number.

The *wing* (Plate III, fig. 1) is broadly lanceolate and about 3.47 times as long as broad (3.44–3.54). In most specimens α is a little shorter than β , while the latter is always greater than γ . The ratio δ over α averaged about 0.43. The wing is about twice the length of θ .

The *hind leg* is about 1.6 times the length of the wing and the femur forms a little less than one-fourth of its length.

The *male genitalia* (Plate III, figs. 9, 10, 11 and 12) resemble those of *P. sylvestris*, *P. malabarensis* and *P. purni*. The basal segment of the *superior clasper* is stout and is almost twice the length of the distal segment. The distal segment carried 4 stout curved spines each about 100 microns long. These spines are arranged in two pairs, one arising from the distal end of the segment and the other pair from a tubercle at about seven-tenths the distance from the base of the segment. The portion of the segment between the two pairs of spines is much narrower than the basal part. The small non-deciduous spine arises slightly proximal to the middle of the segment. The *intermediate appendage* is of the usual type seen in the recumbent-haired group of *Phlebotomus*. It is about one-third longer than the distal segment of the

TABLE I

Phlebotomus arboris (♂)

Structure		Lengths in mms of specimens number —*				Ratios, relative lengths, etc †
		1	2	3	4	
Body	Head and clypeus	0 414	0 400	0 357	0 357	
	Thorax	0 614	0 600	0 543	0 570	
	Abdomen proper	1 343	1 330	1 200	1 185	
	Sup clasper, seg 1	0 339	0 330	0 315	0 330	
	Total length	2 7	2 66	2 40	2 42	
Mouth	Labium	0 243	0 250	0 210	0 235	
	Pharynx, length	0 160	0 162	0 150	0 150	$= 2.95-3.19 \times \text{breadth} \frac{P}{L} = 3.45$
	Pharynx, breadth	0 054	0 051	0 048	0 051	
Antenna	Segment III	0 384	0 384	0 360	0 375	III > IV+V III=XII-XVI
	Segment IV	0 171	0 159	0 150	0 160	
	Segment V	0 156	0 150	0 144	0 160	Formula $\frac{1}{\text{III-XV}}$
	Segment VI	0 150	0 144	0 138	0 153	
	Segments XII-XVI	0 381	0 378	0 363	0 375	
	Total length	1 957	1 943	1 830	1 930	$= 5.0-5.15 \times \text{IIIrd}$ $5.0-5.15 \times \text{XII-XVth}$
Palp	Segment 1	0 040	0 042	0 037	0 042	Formula 1, 2, 3, 4, 5 Rel lengths-2 2 6 3 9 0 10 9 7 $= 1 + 2$
	Segment 2	0 114	0 111	0 100	0 114	
	Segment 3	0 159	0 159	0 138	0 159	
	Segment 4	0 177	0 171	0 156	0 180	
	Segment 5	0 336	0 381	0 285	0 330	
	Total length	0 826	0 864	0 715	0 815	
Wing	Length	1 714	1 714	1 670	1 714	$= 3.43-3.54 \times \text{breadth}, 61 \times \text{leg}$
	Breadth	0 500	0 485	0 485	0 500	$\frac{\alpha}{\beta} 0.070-100 \frac{\beta}{\gamma} 1.06-1.41$
	α	0 314	0 285	0 285	0 307	$\frac{\alpha}{\gamma} 0.73-1.15 \frac{\delta}{\alpha} 0.37-0.54$
	β	0 314	0 371	0 343	0 328	
	γ	0 270	0 343	0 300	0 307	$\frac{\alpha}{\epsilon} 0.66-0.74 \frac{\theta}{\epsilon} 1.90-2.32$
	δ	0 170	0 100	0 121	0 150	
	ϵ	0 443	0 400	0 385	0 420	$\frac{\alpha+\beta}{\theta} 0.72-0.77 \frac{\text{wing}}{\theta} 1.93-2.05$
	θ	0 843	0 870	0 828	0 843	
	\sim	0 043	0 071	0 043	0 071	

TABLE I—concl'd

Structure		Lengths in mms of specimens number —*				Ratios, relative lengths, etc †
		1	2	3	4	
Hind leg	Femur	0.700	0.700	0.643	0.700	$\frac{1}{4}$ leg $2 \times$ tarsus seg. 1 $<$ tarsus segs. 2-5
	Tibia	1.014	1.085	0.970	1.014	
	Tarsus, seg. 1	0.494	0.500	0.464	0.500	
	Tarsus, segs. 2-5	0.585	0.614	0.543	0.614	
	Total length	2.8	2.9	2.6	2.8	
Genitalia	Sup. clasper, seg. 1	0.339	0.330	0.315	0.330	$= 1.93-1.98 \times$ seg. 2, $1.0-1.1 \times$ inf. clasper $= 0.67-0.71 \times$ intermed. append. $= 0.9 \times$ inf. clasper Length protruded $= 1.38 \times$ subgen. lamella
	Sup. clasper, seg. 2	0.174	0.168	0.159	0.171	
	Intermed. appendage	0.243	0.240	0.234	0.243	
	Intromit. organ	0.210	0.195	0.204	0.190	
	Genital filament	0.084	0.180	0.030	0.105	
	Inferior clasper	0.306	0.303	0.291	0.300	
	Subgen. lamella	0.222	0.222	0.210	0.216	

* Measurements of the type and 3 co type specimens † Taken from 8 specimens

superior clasper, but is much shorter than the proximal segment. The *intromittent organ* (Plate III, figs 9 and 10) is slender with a slightly dilated end. It is much shorter than the intermediate appendage. The *genital filaments* were protruded in six out of eight specimens, and in one case measured as much as 180 microns. It shows faint transverse annulations and striations. The *pompetta* lies in the seventh abdominal segment or even more posteriorly. The *inferior clasper* is unarmed but bears a number of long hairs arising from marked tubercles about its distal end. It is about 1.36 times as long as the subgenital lamella and 1.25 times the intermediate appendage but is slightly shorter than the basal segment of the superior clasper.

Differential diagnosis

The absence of erect hairs on the dorsa of segments 2 to 7 of the abdomen, as well as the distribution of the spines on the distal segment of the superior clasper, differentiate this species from the members of the erect-haired group.

The male genitalia are of the type seen in *P. sylvestris*, *P. malabaricus*, *P. squamirostris*, *P. puru* and *P. zeylanicus*, but the following diagnostic characters are present —

1. *P. sylvestris* *—This species has a palpal formula of 1, 4, 2, 3, 5 (♀), α is very much shorter than ρ , the wing is more than 4 times as long as broad, the hind femur forms much less than one-fourth of the leg, the

* This species appears to be identical with *Phlebotomus demeijerei* Nitzulescu, 1930

proximal segment of the superior clasper is relatively long and thin and is much longer than the inferior clasper, the distal segment is much greater than half the length of the proximal one, it is about equal in length to the intermediate appendage, the two pairs of spines are widely separated, the proximal pair arising about the middle of the segment, the non-deciduous spine takes origin between the two pairs of large spines

2 *P malabaricus*— α is more than twice the length of β , the ratio δ over α is more than 0.7, the proximal segment of the superior clasper is relatively thinner and is distinctly longer than the inferior clasper, the two pairs of spines on the distal segment of the superior clasper are closer together and the non-deciduous spine arises between them, the basal portion of the intromittent organ is more expanded than in *P arboris*

3 *P squammosus*—The palpal formula is 1, 2, (3, 4), 5, the pharyngeal armature shows numerous long teeth, the buccal armature consists of a continuous row of about 20 teeth, segment III of the antenna is much shorter than the combined lengths of segments XII to XVI

4 *P puri*—The palpal formula is 1, (2, 4), 3, 5, Newstead's spines number about 15, antennal segment III is much less than the combined length of segments XII to XVI, the total length of the antenna is six times that of segment III, the geniculate spines on the latter segment are relatively shorter, the antennal hairs do not stand out, the wing is about 4 times as long as broad, the teeth of the buccal armature are more marked, the pigmented area is smaller and elongated, the proximal segment of the superior clasper is much narrower and longer, being longer than the inferior clasper by almost one half, the intermediate appendage is about the same length as the distal segment of the superior clasper, the latter segment is of a more uniform width in its whole length, the non-deciduous spine arises between the two pairs of larger spines

5 *P zeylanicus*—The palpal formula is 1, 2, (3, 4), 5, α is more than twice the length of β , the ratio δ over α is more than 0.5, the buccal armature consists of an anterior row of about 10 relatively large teeth separated from each other and usually two or three rows of smaller teeth can be distinguished anterior to these, the pigmented area is small or absent, the intromittent organ seen from the side has a wedge-shaped outline, the proximal pair of spines on the distal segment of the superior clasper arise about its middle, the non-deciduous spine arises between the two pairs of large spines

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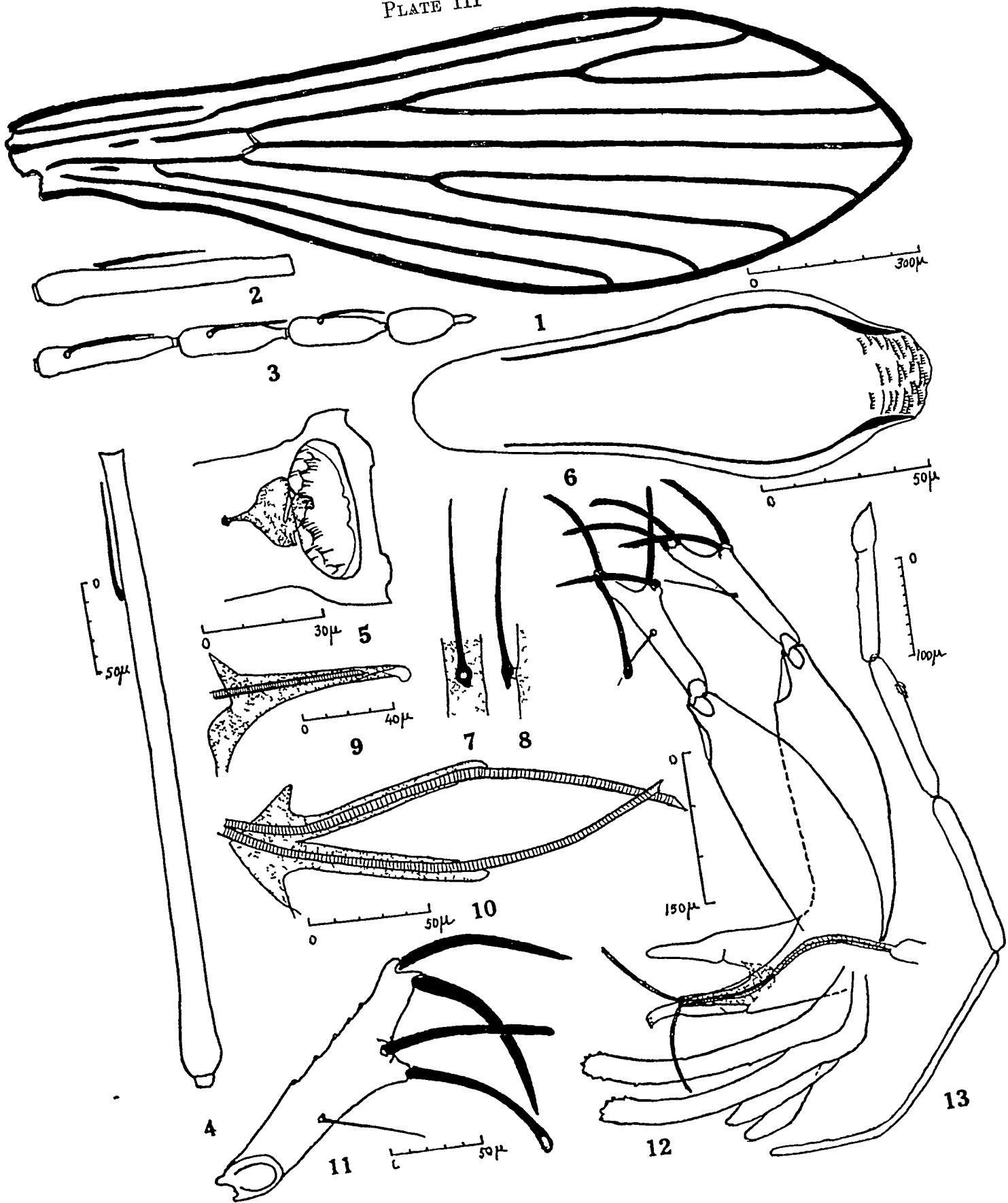
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EXPLANATION OF PLATE III

Phlebotomus arboris (♂)

- Fig 1 Wing
„ 2 Antennal segment IV
„ 3 Antennal segments XIII to XVI
„ 4 Antennal segment III
„ 5 Buccal cavity
„ 6 Pharynx
„ 7 Genuiculate spine seen from above
„ 8 Genuiculate spine seen from side
„ 9 Lateral view of intromittent organ
„ 10 Intromittent organ seen from above
„ 11 Distal segment of superior clasper
„ 12 Genitalia
„ 13 Palp

PLATE III



THE EFFECT OF INTRAVENOUS INJECTIONS OF QUININE ON THE ELECTROCARDIOGRAM IN MAN

BY

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[Received for publication, January 26, 1931]

THE intravenous route for the administration of quinine in the treatment of malaria is usually adopted when a rapid control of the infection is necessary or when the patient is unable, because of severe gastro-intestinal or cerebral symptoms, to take the drug by the mouth. Although this method of administration is preferred by most to intramuscular injection, on account of the risks of local infection, necrosis and even nerve paralysis attendant on the latter procedure, it is not devoid of danger to the patient. Quinine is a circulatory depressant. Cushny (1924) states that, in mammals, quinine given intravenously generally slows and weakens the heart by its direct action on the cardiac muscle and that large amounts may depress the vagus terminations. McCarrison and Cornwall (1919) found that all salts of quinine produce a fall in blood pressure after intravenous injection in sheep, and Brahmachari (1922) showed that a sharp fall in blood pressure follows the rapid intravenous injection of concentrated solutions of quinine in man. These effects of the drug may lead to serious consequences in patients already suffering from circulatory embarrassment, severe toxæmia or the general hyptonia sometimes associated with chronic subtertian malaria.

A study was made by means of the electrocardiograph of the changes produced in the human heart by the intravenous injection of quinine in therapeutic doses. Observations were carried out on hospital patients some of whom were suffering from chronic malaria. With the patient in the semi-prone position control electrocardiographic curves were taken from all three leads and the drug was then slowly injected into the left median basilic vein. Curves were taken from lead II only during and at short intervals after the injection. Nine patients received 6 grains of quinine hydrochloride dissolved in 10 ccs of saline and three 10 grains dissolved in 20 ccs of saline. In two subjects

curves were taken before, during and after control injections of 10 ccs of saline alone. In these individuals the only effect observed was some increase in the heart-rate due to psychic and reflex factors.

RESULTS

1 *Rate of the heart*—Acceleration, greater than that observed during and after saline injections, occurred in all subjects.

2 *The P wave*—In all patients except two this wave was slightly increased in height. In three it was broadened and in two of these (Figs 1 and 5) it was continuous with or superimposed on the preceding T wave.

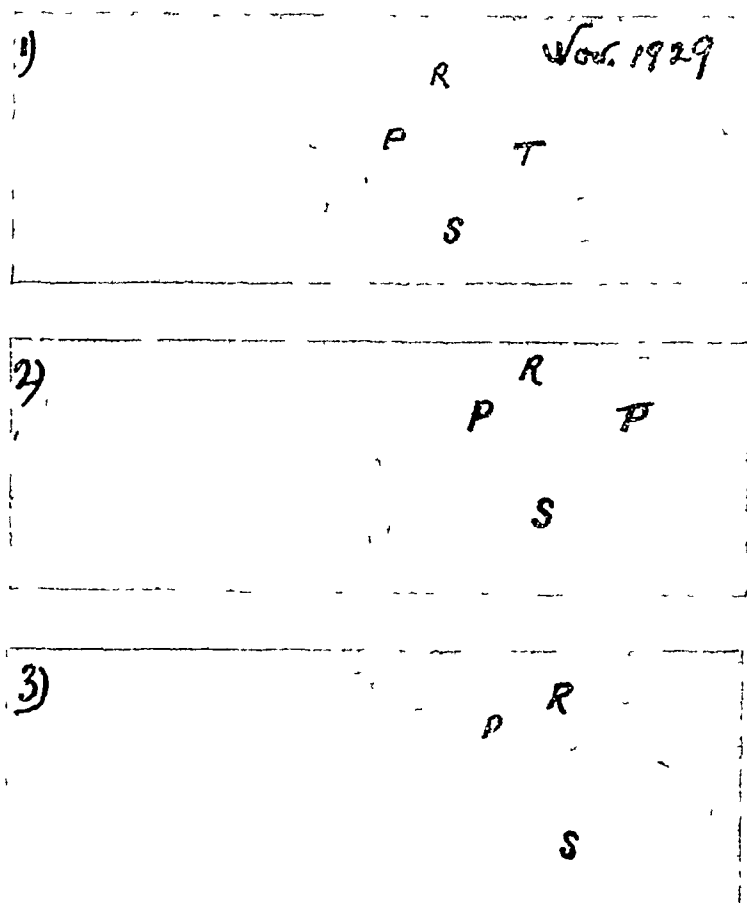


Fig 1—All curves in this and other figures are from lead II. Time is shown in 1/25 sec and height in mm. In originals each 1 cm = 1 millivolt. (1) *Before quinine* Rate 150 per minute, P 1-2 mm, P-R 0.12 sec R 11-12 mm S 3 mm T 0-1 mm. (2) *Immediately after quinine* Rate 176 per minute, P 3 mm P-R 0.12 sec R 10-11 mm S 3 mm P is broadened and continuous with the preceding T. (3) *Half a minute after quinine* Rate 187 per minute, P 3-4 mm P-R 0.12 sec R 10-11 mm S 3 mm P broadened as before.

3 *P-R interval*—In one subject (Fig 2) the P-R interval was shortened from 0.14 sec to 0.04 sec one minute after the injection. The interval

regained its original value in 2 minutes. Slight shortening was seen in 4 other cases.

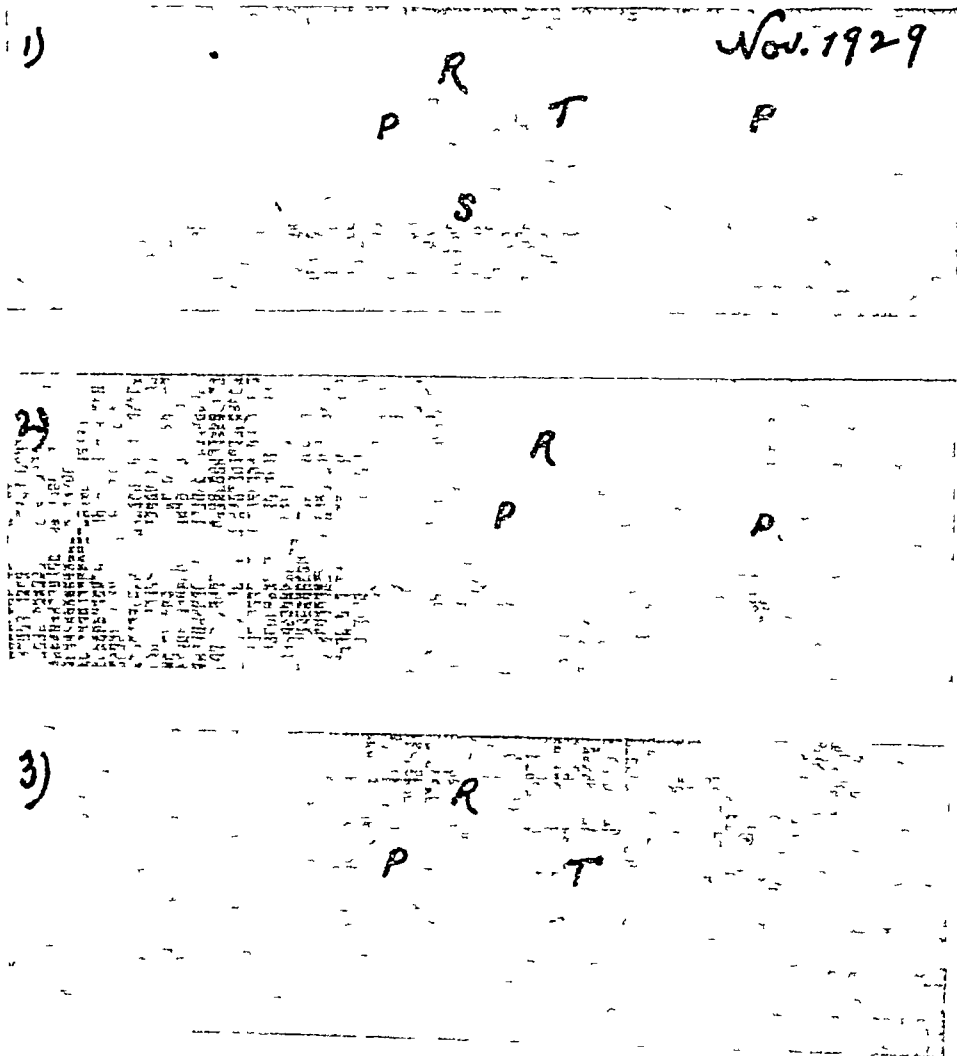


Fig 2—(1) *Before quinine* Rate 81 per minute P 1 mm P-R 0.14 sec R 9 mm S 2-3 mm T 2-3 mm (2) *One minute after quinine* Rate 115 per minute P 1 mm P-R 0.04-0.06 sec R 9 mm S 0-1 mm T abolished (3) *Two minutes after quinine* The curve has almost regained its original form

4 *Ventricular complex*—The T wave was diminished in height or abolished in every case. The R wave was diminished in 6. An increase in height of this wave was not observed. The S wave was increased in height to a small extent in all subjects except 2. Depression of the R-T segment was seen in one patient whose myocardium was defective and in whom the T wave was inverted in leads II and III in the control curves. The injection produced marked electrocardiographic changes in this case (Fig 4).

All the above changes were noticed immediately or soon after injection and as a rule one or more of them lasted 2 to 4 minutes. Significant symptoms

were produced in two individuals only, both of whom showed signs of collapse. In one of these, the patient with myocardial insufficiency, the symptoms were

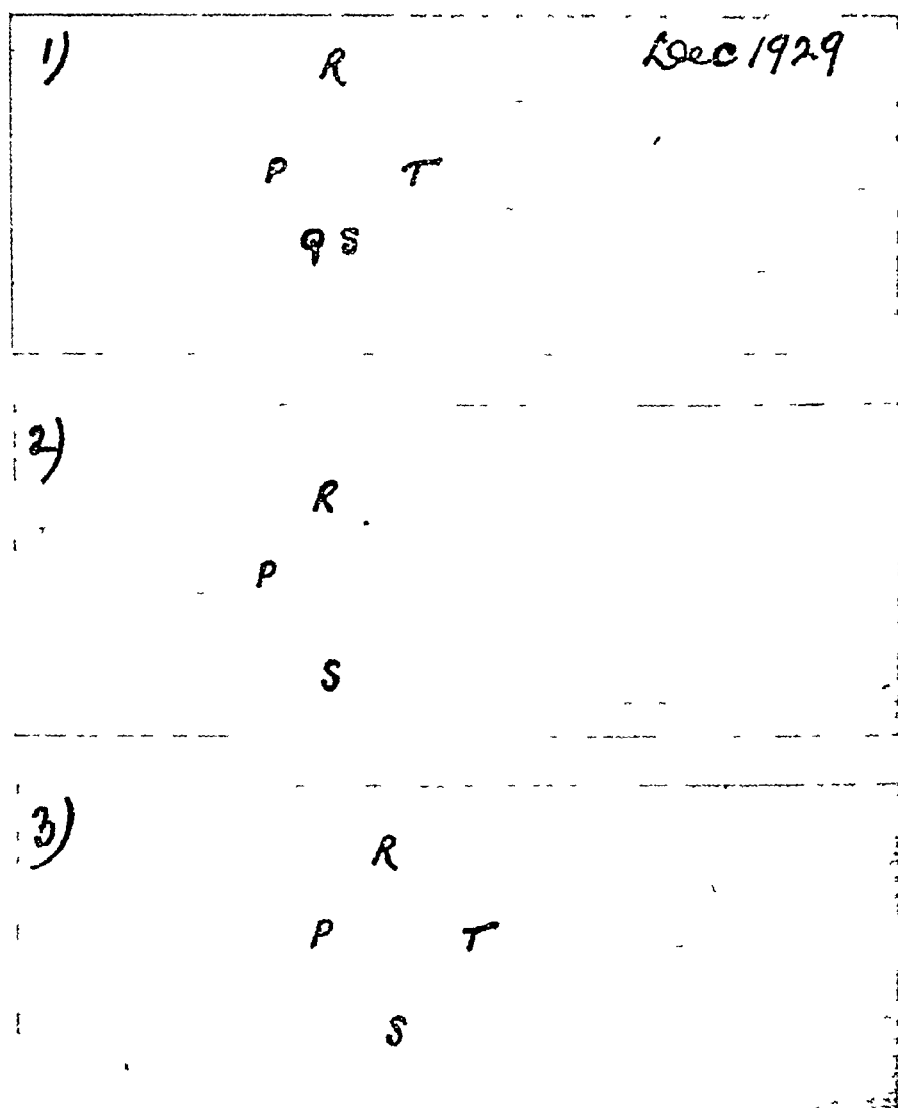


Fig 3—(1) *Before quinine* Rate 115 per minute P 1 mm P-R 0·12 sec R 14 mm S 0·2 mm T 1-1½ mm (2) *Half a minute after quinine* Rate about 130 per minute P 1½ mm P-R 0·10-0·12 sec R 11-12 mm S 3 mm T abolished (3) *Two minutes after quinine* Rate 120-125 per minute P 1½ mm P-R 0·10-0·12 sec R 12 mm S 3 mm T beginning to appear

severe Subcutaneous injection of adrenalin (1 c.c. of 1 in 1,000 solution), however, produced prompt relief and rapidly restored the electrocardiogram to its original form (Fig 4)

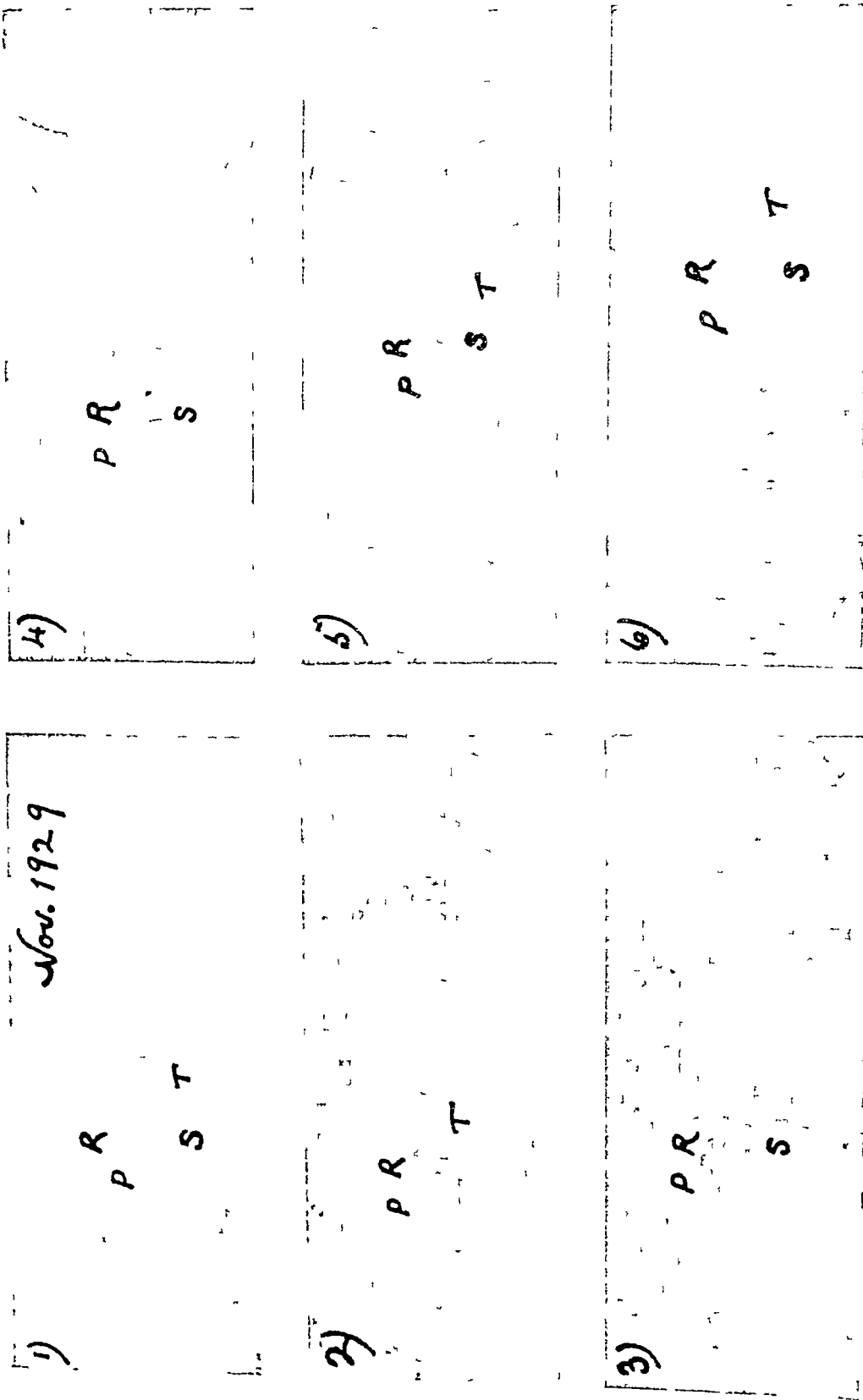


Fig 4—(1) Before quinine Rate 100-107 per minute P 1-1½ mm P-R 0.10 sec R 7-8 mm S 2-3 mm T inverted (2, 3, 4 and 5), 1, 2, 3 and 4 minutes, respectively, after quinine The curve underwent marked changes The rate increased to 166 per minute, P increased in height to 3 mm and became broader, R diminished to 3 or 4 mm, and S increased slightly The R-T segment was depressed after the first minute, T was abolished at first but reappeared in inverted form after 4 minutes The patient showed severe signs of collapse but recovered rapidly after receiving 1 cc of adrenalin (1 in 1,000) subcutaneously The effect of this on the electrocardiogram is seen in curve 6 which was taken ½ minute after adrenalin

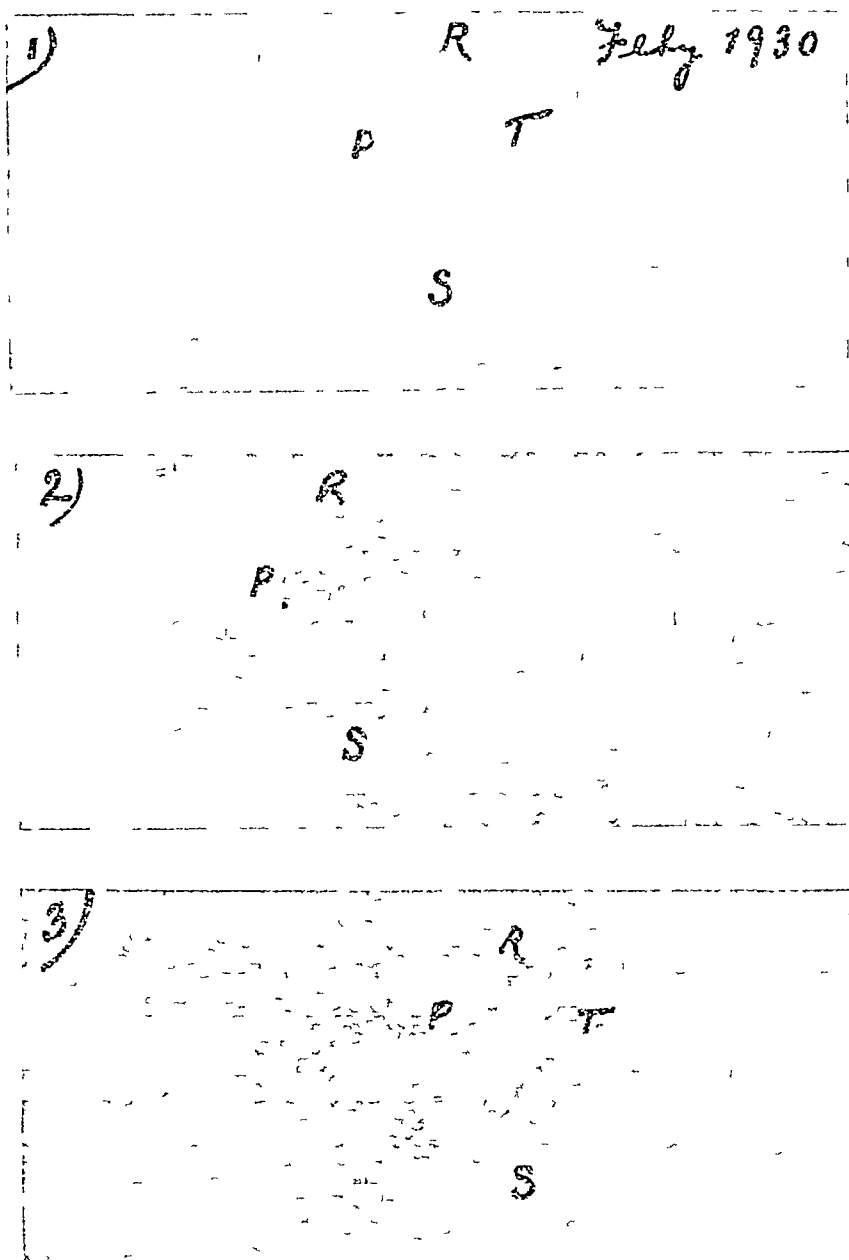


Fig 5—(1) *Before quinine* Rate 100 per minute P 3 mm P-R 0.12 sec R 17-18 mm S 6 mm T 5 mm (2) *Half a minute after quinine* Rate 150-157 per minute P 4-5 mm P-R 0.10 sec R 15-16 mm S 7 mm P is broadened and continuous with the preceding T (3) *Two minutes after quinine* The curve is regaining its original form

DISCUSSION

The alterations in the P wave resemble those that sometimes occur in mitral stenosis, a condition in which there is increased force of auricular contraction. The tachycardia was probably to some extent due to depression of

the vagus as it was much greater after quinine than after saline alone. The slight shortening of the P-R interval observed in 4 cases also indicates diminished vagal tone. The great reduction of this interval shown in Fig 2, however, is similar to that seen when auricular beats derived from the natural pacemaker are followed by 'escaped' ventricular contractions arising in the junctional tissues (Lewis, 1928), and was presumably caused by the direct effect of the drug on these tissues. Of the changes in the ventricular complex the most constant was reduction in height or abolition of the T wave. This was, perhaps, to be expected as the T wave is very unstable, its height, form and direction being influenced even in health by many factors such as emotion, exercise, heat, cold, anoxæmia and digitalis. In most instances, however, other ventricular features besides the T wave were altered and in the patient whose myocardium was deranged severe circulatory collapse was associated with marked changes in the ventricular complex. It may be assumed, therefore, that the alterations in this part of the curve are evidence of the direct depressant effect of quinine on the myocardium. The action of adrenalin in case IV both on the electrocardiogram and on the condition of the patient supports this assumption. Incidentally it also illustrates the advisability of giving adrenalin along with quinine in intravenous injections or at least of having it ready in case of collapse, especially when dealing with patients who show signs or symptoms of cardiac weakness.

CONCLUSIONS

The changes produced in lead II of the electrocardiogram by the intravenous injection of quinine in anti-malarial doses were studied in 12 patients. The most constant effect was a reduction in height or abolition of the T wave. Other effects observed were tachycardia, increase in height and width of the P wave, shortening of the P-R interval, reduction in height of the R wave and increase of the S wave. In one individual the P-R interval was reduced to 0.04 sec. Collapse, which was promptly relieved by adrenalin, occurred in two cases, in one of whom there was inversion of the T wave in leads II and III in the control curves. Symptoms were severe in this patient and were accompanied by marked electrocardiographic changes including depression of the R-T segment.

The electrocardiograms were taken by my Research Assistant Dr D L Shrivastava, DSc, to whom my best thanks are due. I am also indebted to Dr Mohammad Yusaf, M.D., my Clinical Assistant, and Dr B S Bhandari, my House Physician, for assistance during the investigation.

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EFFECT OF EMETINE ON BLOOD-SUGAR

BY

N K BASU, M B

[Received for publication, January 29, 1931]

Introduction

THOUGH emetine has been used for the past several years very successfully against *E histolytica* and with occasional successes against hæmorrhage and hepatic insufficiency, its pharmacological actions have not been yet fully worked out. Chopra (1927 and 1928) from the Calcutta School of Tropical Medicine has worked out a portion of the pharmacological action of this drug. Emetine has a marked action on the liver and one of the functions of the liver being carbohydrate metabolism, it was decided to test the change, if there be any, in the amount of sugar present in the blood after injection of emetine.

To a series of animals (rabbits and cats) both intramuscular and intravenous injection of emetine showed decided hyperglycæmia, which did not last for a long time. This transitory character of hyperglycæmia led to the suspicion of temporary stimulation of the vagus with this drug, which was corroborated in the atropine experiment. When atropine is injected previously to paralyse the vagal nerve endings, emetine fails to produce this hyperglycæmia.

Vagal hyperglycæmia

The influence of the vagus and of certain drugs in the production of hyperglycæmia is a well-known fact. But this nervous excitation can explain only transitory increase in blood-sugar.

About the relation of the vagus to the islets of Langerhan there is a great deal of controversy. Hoshi (1926) and La Baire (1927) showed that after stimulation of the vagus there is increased insulin content in the blood and consequently the blood-sugar falls. But against these findings there are evidences to prove that the action of the vagus on the islets of Langerhan is inhibitory rather than secretory. Moreover, Zung and La Baire (1927)

showed that after injection of adienalin, though there was increase of insulin in the pancreatic vein blood, blood-sugar always increased

Animal experiment

To test the effect of emetine on blood-sugar, a series of experiments was carried out on animals (rabbits and cats). As a rule, rabbits are very susceptible to emetine, particularly when it is injected intravenously. So one has to be very cautious about the dose of emetine when rabbits are used for the experiment.

Control of diets and habits

As a slight change in the diets causes a great variation in the percentage of blood-sugar, all these experiments were done on practically starved animals. They were given the same kind and same amount of food once a day at a fixed time and they were kept in separate cages while the experiments were carried out. Samples of blood were collected every day at the same time and before the food was given. Samples were not collected before the animal was absolutely calm and quiet.

General effect of emetine on blood-sugar

From Tables I, II, III and IV, it is evident that after injection of emetine blood-sugar always increases. This rise of blood-sugar does not last for a long time and is not proportional to the amount of the drug injected.

To determine whether this rise in blood-sugar is due to stimulation of the supra-renal glands, two methods were adopted. In the first method (Table VI-a) the glands were stimulated by injecting 0.2 cc of adienalin chloride solution (1 in 1,000) subcutaneously into a rabbit and after some time when the blood-sugar reached a constant level 1 mg of emetine was injected through the ear vein. Still the blood-sugar showed a definite and marked rise. In the second method (Table VI-b) the supra-renal vessels were clamped but still emetine caused a well marked rise. But the blood-sugar falls very quickly. So the rise in the blood-sugar is not due to stimulation of the supra-renal glands but at the same time it is very probable that the supra-renal glands are involved in some way in the maintenance of the higher level of blood-sugar after emetine, though this is not for long.

Nearly half of the amount of glucose in the blood comes from the muscles. To find out whether this increase of blood-sugar after emetine was due to increased production in the muscles, limbs of cats were perfused with Locke's solution which was oxygenated and mixed with defibrinated blood. When the colour of the perfused fluid began to approach that of the perfusion fluid, the glucose content was measured and when it was constant emetine was injected. The glucose content was measured afterwards and was found to be the same.

as before the injection of emetine (Table VII) So the increase in the blood-sugar after emetine is not due to any increased production in the muscles

To find out whether the vagus nerves are involved in any way atropine was injected in sufficient amount to paralyse the vagal endings Emetine was then injected and so long as the effect of atropine lasted there was no rise of blood-sugar (Table V) In the other three experiments of the same group, the blood-sugar only went up when the effect of atropine had passed off Thus it is evident that the rise in blood-sugar after emetine is a nervous phenomena and is due to stimulation of the vagus nerves

It has been said before that the action of the vagus on the islets of Langerhan is inhibitory rather than secretory Now a fixed amount of insulin is always secreted and present in the blood which keeps the level of glucose content constant So there is an inverse relation between the concentration of insulin and glucose in the blood To determine whether this stimulation of the vagus by emetine affects in any way the secretion of insulin, the pancreatic vessels and ducts were all ligatured Then emetine was injected, but the blood-sugar did not rise, practically remaining in the same level as before injection So it is evident that emetine stimulates the vagus nerve supply to the pancreas and the nerve being inhibitory to the pancreatic gland the secretion is diminished and correspondingly the sugar content of the blood goes up

Methods followed for the estimation of blood-sugar

Colorimetric method of Folin-Wu has been followed throughout the experiment But very often results were corroborated by Maclean's method as well

Choice of anæsthesia

Almost every anæsthesia causes a variation in the glucose content of the blood, and amytal is said to be the only substance which does not affect the blood-sugar But the variation caused by different anæsthesias was once more tested during the course of this experiment It was found that amytal does not affect the blood-sugar Urethane, ether and chloralose cause a rise in the blood-sugar But it was found that after a period of three hours the blood-sugar attained a constant level and remained constant at that level for at least two hours Clark has used amytal and ether anæsthesia in all his experiments and he too expresses the same opinion Throughout the present experiment amytal has been chiefly used, the first experiment from Table IV and the first experiment from Table V were done under chloralose anæsthesia, and in experiment under Table III ether was used But in these three experiments blood-sugar was not tested before it attained a constant level

CONCLUSIONS

- (1) Emetine raises the blood-sugar
- (2) Emetine stimulates the vagus—as shown by atropine experiments—which is the cause of the rise of the blood-sugar

(3) Emetine does not affect the secretion of the supra-renal glands

(4) Emetine does not seem to affect the glycogenolytic process in the liver or in the muscles

(5) Emetine seems to inhibit the secretion of the islets of Langerhan through the inhibitory fibres of the vagus

ACKNOWLEDGMENT

I beg to acknowledge with thanks the valuable suggestions received from my old chief, Lieut-Colonel R N Chopra, M A, M D, I M S, of the Calcutta School of Tropical Medicine, during the course of this experiment

TABLE I

No	Animal used	Weight	Emetine inj per kilo	BLOOD SUGAR PERCENTAGE BEFORE AND 24 HOURS AFTER EMETINE	
				Before	After
1	Rabbit	1 6 kilo	5 mg	0 102	0 12
2	"	1 7 "	"	0 166	0 157
3	"	1 75 "	"	0 105	0 12
4	"	1 8 "	"	0 097	0 11

These animals were not given any anæsthesia. Blood was drawn by heart puncture and injection of emetine was made intramuscularly

TABLE II

No	Animal used	Weight	Total amount of emetine injected	BLOOD SUGAR PERCENTAGE BEFORE AND 30 MINUTES AFTER EMETINE	
				Before	After
1	Rabbit	1 4 kilo	1 0 mg	0 135	0 17
2	"	1 6 "	1 1 "	0 124	0 15

These animals were not given any anæsthesia. Blood was drawn from the ear vein and injection of emetine was made through the same channel

TABLE III

No	Animal used	Weight	Total amount of emetine injected	BLOOD SUGAR PERCENTAGE BEFORE AND 30 MINUTES AFTER FMETINE	
				Before	After
1	Rabbit	1.6 kilo	10 mg	0.129	0.166

Under light anaesthesia the femoral vein of the animal was exposed and blood was drawn and emetine injected through the same channel

TABLE IV

No	Animal used	Weight	Total amount of emetine injected	BLOOD SUGAR PERCENTAGE BEFORE AND AT VARIOUS TIMES AFTER EMETINE				
				Before	15 min	30 min	45 min	60 min
1	Cat	1.8 kilo	10 mg	0.07	0.11	0.18		0.16
2	„	2.7 „	3 „	0.08	0.10	0.125	0.148	
3	„	2.5 „	4 „	0.07	0.083	0.10		0.114

Under complete anaesthesia the femoral vein of the animals were exposed and injection of emetine was given through the same channel. After injection blood was collected at the interval of every 15 minutes

TABLE V

No	Animal used	Weight	Total amount of emetine injected	BLOOD SUGAR PERCENTAGE BEFORE AND AFTER ATROPINE, AND AT VARIOUS TIMES AFTER EMETINE					
				Before	After atropine	15 min	30 min	60 min	135 min
1	Rabbit	1.7 kilo	1 mg	0.14	0.175	0.172	0.174	0.17	
2	Cat	2.2 „	4 „	0.088	0.105	0.105	0.105	0.107	0.15
3	Rabbit	1.5 „	1 „	0.14	0.168	0.166	0.169	0.17	0.19
4	Cat	2.4 „	4 „	0.09	0.11	0.108	0.115	0.12	0.15

Under complete anaesthesia the femoral vein was exposed. One sample of blood was first collected and then atropine was injected. After 15 minutes another sample of blood was collected. Then emetine was injected and blood was collected at the interval of every 15 minutes.

TABLE VI-a

No	Animal used	Weight	BLOOD SUGAR PERCENTAGE BEFORE AND AFTER ADRENALIN AND EMETINE		
			Before	After adrenalin	After emetine
1	Rabbit	1.5 kilo	0.145	0.22	0.3

The animal was not given any anaesthesia. Blood was drawn by heart puncture. Adrenalin was injected subcutaneously. Then 1 mg of emetine was injected through the ear vein.

TABLE VI-b

No	Animal used	Weight	BLOOD SUGAR PERCENTAGE BEFORE AND AFTER CLAMPING AND AT VARIOUS TIMES AFTER EMETINE			
			Before	After clamping	30 min	60 min
1	Cat	2.1 kilo	0.08	0.12	0.22	0.10

Under complete anaesthesia the femoral vein was exposed and abdomen was opened. Blood samples were collected before and after clamping the supra-renal vessels. Then emetine was injected and blood was collected after an interval of 30 minutes.

TABLE VII

No	Total amount of emetine injected	GLUCOSE CONTENT BEFORE AND AT VARIOUS TIMES AFTER EMETINE		
		Before	15 min	60 min
1	2.0 mg	0.166	0.16	0.158
2	4.0 "	0.174	0.17	0.177

Under complete anaesthesia limb of cats was perfused. When the colour of the perfused fluid approached the colour of the perfusion fluid, its glucose

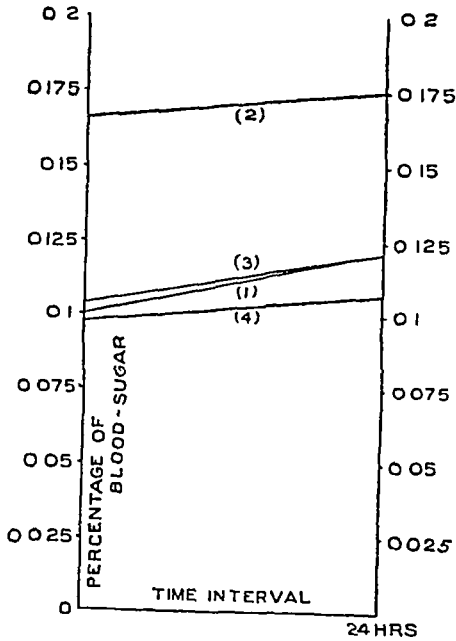
content was estimated. Then emetine was injected and samples of perfused fluid were tested at various intervals.

TABLE VIII

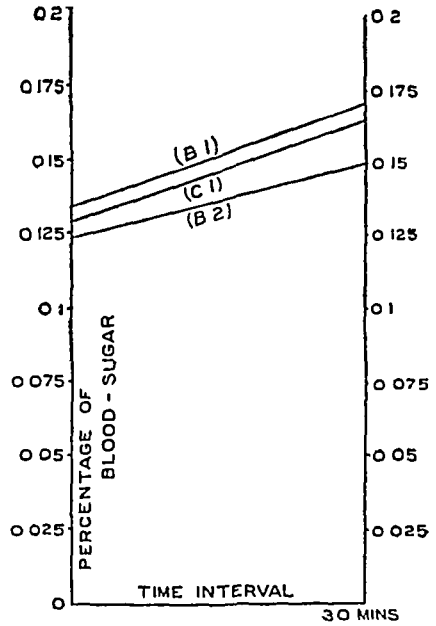
No	Total amount of emetine injected	BLOOD SUGAR PERCENTAGE BEFORE AND AFTER LIGATURE AND AT DIFFERENT TIMES AFTER EMETINE			
		Before	After ligature	30 min	60 min
1	4.0 mg	0.08	0.083	0.083	0.078
2	4.0 „	0.092	0.096	0.094	0.09

Under complete anaesthesia pancreatic vessels and ducts were ligatured. Blood samples were collected before and after ligature. Then emetine was injected and two samples were collected again at the interval of 30 minutes.

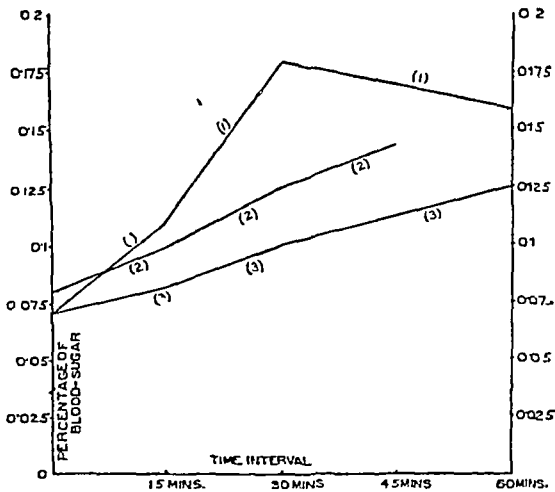
GRAPH (I)



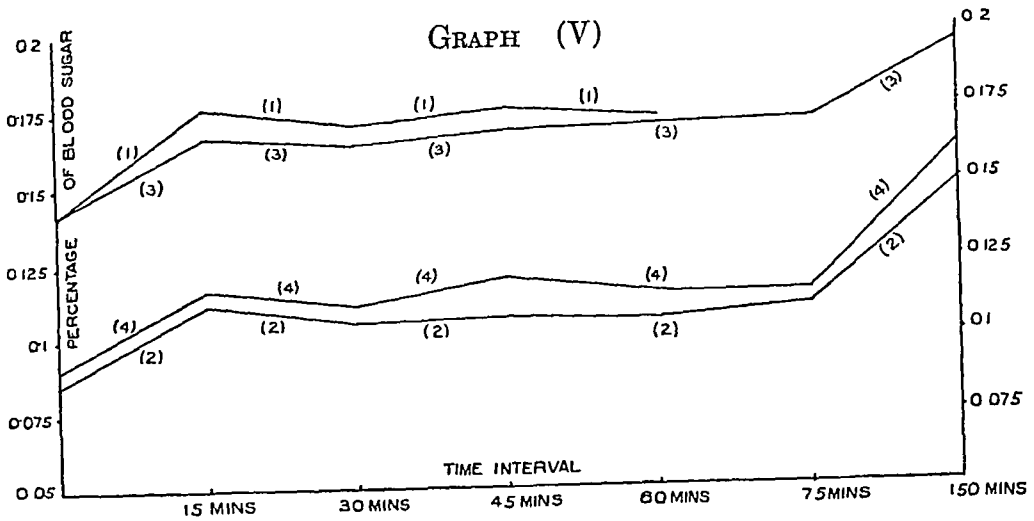
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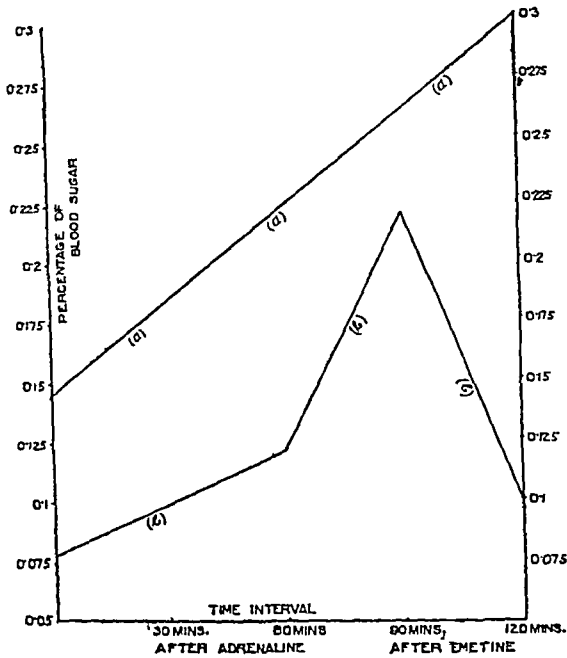
GRAPH (IV)



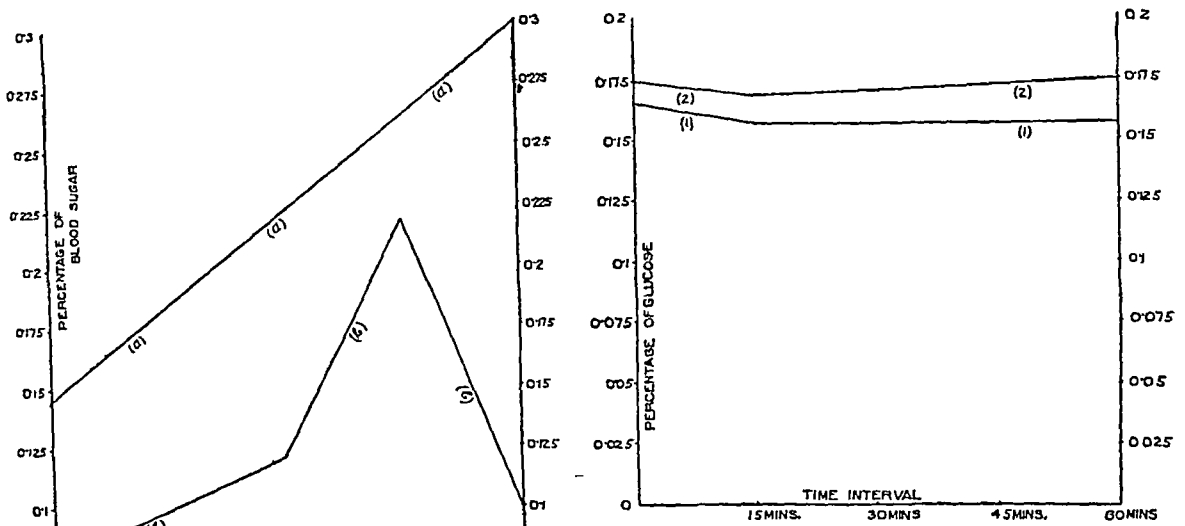
Note—The Graphs are given numbers corresponding to their respective Tables



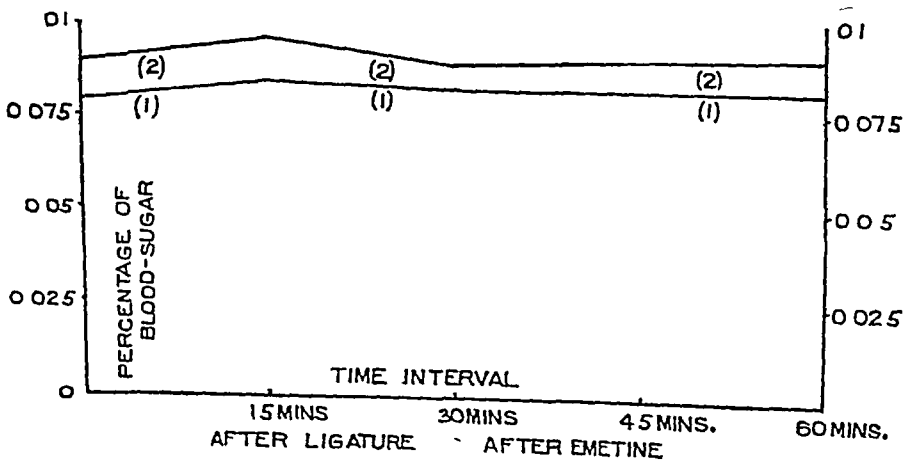
GRAPH (VI)



GRAPH (VII)



GRAPH (VIII)



Note—The Graphs are given numbers corresponding to their respective Tables J, MR

PRELIMINARY OBSERVATIONS ON THE INFLUENCE OF
DIFFERENT CONCENTRATIONS OF HYDROGEN IONS,
AND TEMPERATURES OF WATER ON MOSQUITO
LARVÆ (*ANOPHELES SUBPICTUS*) *

BY

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[Received for publication, February 3, 1931]

SINCE the end of the last century when it was discovered that the Anopheline mosquito is responsible for the spread of malaria, efforts have been made to devise means by which the mosquito or its larvæ, the potential transmitters of the disease, can be kept under control. That a detailed and careful study of the habits and habitat of an animal is the most essential desideratum before means can be devised for keeping it under check is a truism. In the case of the mosquito, however, such elementary but important questions as the nature of the food of the larvæ or their behaviour under different conditions of environment are not yet definitely settled. With regard to the influence of environment, some time ago I took some observations on the effect of different temperatures and the concentrations of hydrogen ions of water on the development of *Anopheles subpictus*. The hydrogen-ion concentration or active acidity of a water in view of its profound influence, both direct and indirect, on the biology of aquatic organisms is widely recognized to be a reliable index to the suitability of the water as a habitat for different animals. Many animals flourish best in a limited range of this factor. To illustrate this statement I may quote the familiar case of hay infusions in which certain Protozoa appear and disappear in a regular sequence. A few years ago I showed† that the hydrogen-ion concentration of a hay infusion undergoes a series of changes and that different Protozoa appear and flourish when the

* Read at the 18th Session of the Indian Science Congress, Nagpur

† *Brit Jour Exper Biol*, 1927

concentration is suitable for their existence. In reference to mosquito larvæ, no doubt there are numerous observations on record indicating the various temperatures and hydrogen-ion concentrations at which the larvæ have been found to occur, but hardly any author has touched on the important question whether such temperatures and acidities are optimum for the development of the larvæ into pupæ and adults. If some of them are not, then waters having such condition though teeming with the larvæ need not bother either the entomologist or the Public Health Officer, as there will be very little chance for the larvæ to become adult and so to transmit disease. It is simply to call attention to this aspect of the problem that the following observations are described.

Some of the observations on the influence of different concentrations of hydrogen ions are detailed in Table I. By the addition of sodium bicarbonate and HCl to pond water six different concentrations were prepared into each of which 15 larvæ of the same age were introduced. Daily records were made of the number and condition of the larvæ found living. Bodies of dead individuals were removed every morning. An examination of the table will show that with the exception of the highest concentration tried (pH 3.5), at which all the larvæ died within 3 days, the larvæ apparently flourished equally well when kept at all other concentrations, viz., from pH 5.2 to 9.8. But the number of larvæ that metamorphosed in the different jars was very different. Of those kept at pH 5.2, 9 pupated of which only 2 became adult. Of all the concentrations tried, the pH 7.4 seems to be most suitable for the metamorphosis of the larvæ, as at this pH nine pupated, all of which became adult. At pH 8.5 five pupated of which four became adult. At pH 9.0 only one pupated and reached the adult stage, whereas at pH 9.8 all the larvæ died without undergoing metamorphosis. Thus it is evident that though the larvæ can live in a wide range of pH, they can only become pupæ and adults in a very limited range of this factor.

Three sets of observations on the influence of different temperatures are detailed in Table II. In each experiment 15 larvæ were kept at a high temperature, varying from 32°C to 34°C, while the same number of larvæ of the same age were kept at a lower temperature varying from 28°C to 30°C to serve as control. As in the experiments on the influence of different hydrogen-ion concentrations, daily records were made of the number and condition of the larvæ found living. An examination of the table will show that whereas some larvæ can live at the high temperatures named above, very few indeed can become pupæ and adults at such temperatures. In each of the three experiments detailed in the table only one larva out of 15 kept at the high temperature pupated and out of the three pupæ only one became adult. In the controls 6 to 10 out of 15 pupated and a great majority of them reached the adult stage.

It is highly probable that in nature *Anopheles subpictus* will be found breeding freely in water with temperature 32°C–34°C or even higher in the

TABLE I
Influence of different concentrations of hydrogen ions of water on the larvæ of Anopheles subpictus

Period in days	15 larvæ at pH 3.5	15 larvæ at pH 5.2	15 larvæ at pH 7.4	15 larvæ at pH 8.5	15 larvæ at pH 9	15 larvæ at pH 9.8
1	12 larvæ	15 larvæ	15 larvæ	15 larvæ	15 larvæ	15 larvæ
2	8 " (all in-active)	"	14 "	"	"	"
3	2 " (all in-active)	"	"	14 "	"	"
4	Nil	11 " and 2 pupæ	13 " and 1 pupa	7 " and 3 pupæ	11 "	13 "
5		3 " 7 pupæ and 2 adults (removed)	5 " 1 pupa and 6 adults (removed)			
6		Nil	2 " 2 pupæ and 1 adult (removed)	3 " 2 pupæ and 3 adults (removed)	8 " and 1 pupa	9 "
7			1 larva and 2 adults (removed)	1 larva and 1 adult (removed)	3 " and 1 adult (removed)	6 "
8	None pupated or became adult	9 pupated of which 2 became adult	9 pupated and became adult	5 pupated of which 4 became adult	1 pupated and became adult	None pupated or became adult
			Nil	Nil	Nil	Nil

TABLE II
Influence of different temperatures of water on the larvæ of Anopheles subpictus

Period in days	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3	
	15 young larvæ at 32°-34°C	15 young larvæ at 28°-30°C	15 fully grown larvæ at 33°C	15 fully grown larvæ at 28°-30°C	15 fully grown larvæ at 32°-34°C	15 fully grown larvæ at 28°-30°C
1	14 larvæ	14 larvæ	13 larvæ and 1 pupa	10 larvæ and 2 pupæ	12 larvæ	15 larvæ
2	10 "	12 "	" and 1 adult (adult removed)	9 " 1 pupa and 2 adults (removed)	8 " and 1 pupa (dead)	"
3	9 "	12 "	11 larvæ	5 " 4 pupæ and 1 adult (removed)	6 "	9 " and 3 pupæ
5	5 "	8 "	1 pupa and 1 adult (removed)	1 larva and 7 adults (removed)	1 larva	1 larva, " " and 4 adults (removed)
6	3 "	7 "	1 pupa and 1 adult (removed)	Nil	Nil	Nil
7	3 "	6 "	1 pupa and 1 adult (removed)			
9	2 "	5 "	and 2 adults (removed)			
12	1 larva and 1 pupa (small)	All died due to fungus growth in the water				
14	One pupated, none became adult	Six pupated out of which 5 became adult	One pupated and became adult	Ten pupated and reached the adult stage	One pupated, none became adult	Six pupated out of which 4 became adult

day time Of course such a condition is not at all comparable to that inside a thermostat Apart from other factors, the temperature itself while almost constant inside a thermostat greatly fluctuates in nature at different times of the day, being invariably lower during night time

To conclude, the observations just now described indicate that though mosquito larvæ can live in a wide range of temperature and hydrogen-ion concentration, they can become pupæ and adults under much more limited conditions It is highly probable that the same holds good in the case of other factors of environment This means that even if a certain locality has mosquito larvæ, it may not have adult mosquitoes As it is only the adult mosquito that spreads malaria, whereas the method in vogue for keeping it under check consists of killing its larvæ, it is suggested that before spending money and time on destroying the larvæ in a certain water, it will be economical to ascertain if the condition of the water will allow the larvæ to reach the adult stage

I desire to express my thanks to Mr M O T Iyengar of the Public Health Department, Bengal, who kindly supplied me mosquito larvæ for the experiments described above

THE BEHAVIOUR OF BACTERIOPHAGE PRESENT IN RAW WATER IN THE PROCESS OF SAND FILTRATION

BY

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[Received for publication, February 6, 1931]

It is now an accepted fact that bacteriophage is constantly found in polluted water. But whether it plays any part in the purification of water has been a controversial point. Some among whom may be mentioned Ailong (1926), Arnold (1925), and Nakasima (1925), believe that bacteriophage plays an important part in the self-purification of water, whereas Flu (1923), Zdansky (1924) and Janey (1927) think that it has no such action in natural conditions. If the contention of the workers who hold that bacteriophage destroys bacteria in water be true, then it must be of equal interest to see how the lysin would behave in the purification of raw water in sand filters.

Arnold considered that impregnation of water filters with bacteriophage active against certain pathogenic gastro-intestinal bacteria might offer a new field of investigation in water purification problems. Nakasima studied the effect of intermittent surface filters and sprinklers on bacteriophage present in domestic sewage. He observed that phage originally present in the sewage was unaffected by both the processes, though the chemical purification was quite good. This led him to believe by analogy that bacteriophage may play some rôle in the purification process by sand filters.

The present work was taken up to elucidate the following points —

(1) Whether bacteriophage originally present in raw water takes any part in the diminution of bacteria present in raw water by sand filters

Consideration of this question is important from a sanitary point of view. If it is found to be the case then it opens up a new avenue in the water purification problems and this further knowledge may be utilized by sanitarians in improving sand filtration processes.

(2) To make a comparative study of the activity of bacteriophage in raw water, in settled water and in filtered water, to find out whether it is unaffected by the filtration process or undergoes diminution like bacteria. Investigation on this point seems to us to be of interest on the following considerations —

D'Herelle (1926) claims that bacteriophage plays an important part in the production of recovery from and protection against infectious intestinal diseases and if the lytic action of phage which is constantly present in raw water remains undiminished in the filtered water, it would be disseminated throughout the whole population through drinking water and might thus play an important rôle in the prevention of gastro-intestinal diseases.

METHOD OF INVESTIGATION

The following technique was followed throughout our experiment for the isolation of bacteriophage —

Fifty c.c. of water from each source was put in a flask to which 5 c.c. of 10 per cent nutrient broth (pH 7.8) was added and kept in the incubator for 18 hours at 37°C. It was then filtered through filter paper coated with infusorial earth and finally passed through a L_3 candle in Martin's filtering apparatus. The filtrates were kept in the incubator overnight for testing sterility and then kept in a cool room till used.

(1) *Rôle of bacteriophage in sand filtration process*

For investigation of this question two slow sand filters were taken up which we will refer to as No. I and No. II. In the raw water of No. I strong anti-coli phage active against *B. coli* isolated from raw water was constantly found to be present and in the raw water of No. II it was absent. We then made a comparative study of the efficiency of these two filters in relation to their power of reduction of bacteria present in raw water. For these purposes bacteriological examination was made of three samples from each plant, one from the raw water, one from the settled water, and another from the filtered water. Clemesha's method was followed.

Out of a series of experiments conducted, the result of one is given below in Table I.

It will be evident that plant No. II where phage is absent has given a better bacteriological result. In our series of experiments we observed that reduction of bacteria by slow sand filters was independent of the presence or otherwise of bacteriophage in raw water.

TABLE I

	<i>B coli</i> PRESENT IN		
	Raw water	Settled water	Filtered water
Plant No I (anti coliphage present)	0 0001 c c	0 1 c c	20 c c
Plant No II (anti coliphage absent)	0 0001 c c	0 1 c c	30 c c

(2) *Comparative study of lytic action of bacteriophage in raw, settled and filtered water*

In this case experiments were conducted both in slow and rapid sand filters where active phage was constantly present in the raw water

For the purpose of comparison three samples from each waterwork were examined for lytic action, one from the raw, one from the settled and another from the filtered water

The method of comparison of lytic action of the three different sources of water mentioned above in each case was as follows —

Twenty-four hours' broth cultures of organisms to be tested were made. The following organisms were tested in each experiment (1) *B coli communis*, (2) *B shiga* and *B flexner*, (3) Cholera vibrio, and (4) *B typhosus*. Four sets of broth tubes were then arranged. Two drops of each culture was inoculated into 6 tubes in each set. One was set aside as a control and to the remaining five tubes in one set filtrate of raw water was added in progressive dilution, the first one receiving 1 c c and the 5th tube 1/10,000 c c of the filtrate. To the second and third set was added the filtrate of settled and filtered water respectively exactly in the same manner. The broth tubes were then placed in the incubator at 37°C and examined for lytic action after 18 hours.

Results obtained in slow sand filters and in rapid sand filters differed. The results are therefore dealt with separately.

(A) *Results of experiments with slow sand filters*

Experiments were conducted with several plants. The results obtained were consistent so it will be convenient to describe the work done in one plant, the slow sand filter at Palta, from which water is supplied to the city of Calcutta.

Raw water is drawn here from the river Ganges and we have on every occasion found potent bacteriophage in it active against one or other of the organisms mentioned above.

No alum was being added to the raw water at the time we conducted our experiments and the period of settlement was about three days

Experiments were carried out on seven consecutive days One of the results is given below in Table II —

TABLE II

Name of organisms	Type of water	Amount of bacteriophage added in c c				
		1	1/10	1/100	1/1,000	1/10,000
<i>B coli communis</i>	A B C	++++ ++++ —	++++ ++++ —	++++ ++ —	++++ — —	
<i>B shiga</i>	A B C	++++ ++++ —	++++ ++++ —	++++ — —	++++ — —	
<i>B flexner</i>	A B C	++++ ++++ —	++++ ++++ —	++++ — —	++++ — —	
<i>V cholerae</i>	A B C	— — —	— — —	— — —	— — —	
<i>B typhosus</i>	A B C	— — —	— — —	— — —	— — —	

A = Raw water, B = Settled water, C = Filtered water

++++ = Complete lysis

++ = Moderate lysis

— = No lysis

It will be seen from the table that there is a marked reduction of virulence of bacteriophage by settlement alone In our series of experiments this reduction was between 90 to 99 per cent In the filtered water we were unable to detect the presence of bacteriophage To determine whether the phage was completely destroyed or not we submitted a few filtrates of filtered water to a few passages In some cases the bacteriophage was recovered, in others we failed to recover it showing that it had completely disappeared

(B) Experiments with rapid sand filtration

Ten filters in different locality were examined The source of raw water in these filters is the river Ganges

Alum is added to the raw water as it enters the coagulating tank in these types of filters, and only a few hours settlement is allowed

At the outset we investigated whether the addition of alum up to 4 parts per 100,000 had any deleterious action on the activity of bacteriophage and found that it had none

As alum reduces the alkalinity of water, we also examined the pH of samples of water from the settling tank. It was found to vary from 7.4 to 7.8

Of a series of experiments conducted the result of one is given below in Table III

TABLE III

Name of organisms	Type of water	Amount of bacteriophage added in c.c.				
		1	1/10	1/100	1/1,000	1/10,000
<i>B. coli communis</i>	A	++++	++++	++++	++	—
	B	++++	++++	++	—	—
	C	++	—	—	—	—
<i>B. shiga</i>	A	++++	++++	++++	++++	—
	B	++++	++++	++++	—	—
	C	++++	++	—	—	—
<i>B. flexner</i>	A	++++	++++	++++	—	—
	B	++++	++++	++++	—	—
	C	—	—	—	—	—
<i>V. cholerae</i>	A	—	—	—	—	—
	B	—	—	—	—	—
	C	—	—	—	—	—
<i>B. typhosus</i>	A	++++	++++	—	—	—
	B	++++	++++	—	—	—
	C	—	—	—	—	—

A = Raw water, B = Settled water, C = Filtered water

++++ = Complete lysis

++ = Moderate lysis

— = No lysis

It will be seen from the table that reduction of virulence of phage is not the same in all the organisms. In the settled water there is 90 per cent reduction of activity against *B. coli* and *B. shiga* but the virulence against *B. typhosus* and *B. flexner* remains unimpaired. In the filtered water also reduction is variable, phage being absent in 1 c.c. against *B. typhosus* and *B. flexner* and present in 1 c.c. and 1/10 c.c. against *B. coli communis* and *B. shiga* respectively.

Viewing the result of series of experiments conducted, it may be stated that by settlement alone after the addition of alum, we obtained on an average 90 per cent reduction but activity against some of the organisms remained unaffected. In the filtered water there was a relative reduction of virulence against all the organisms but the proportion varied. Ninety to ninety-nine per cent reduction of virulence of phage against all the organisms used occurred, while the activity of some of the strains disappeared.

DISCUSSION

In a previous paper (Stewart and Ghosal, 1930) we showed that the activated sludge process has a destructive action on sewage bacteriophage. As a result of the present work we found that sand filters also have a similar action on phage present in raw water. The behaviour of bacteria and phage present in raw water is the same in the purification process by sand filters, both undergo diminution in the same manner. Our results are not quite in agreement with the finding of Nakasima, who working with domestic sewage found that phage was unaffected by the process of purification by sand filters. It is difficult to account for his observation, it is probable that the virulence of phage he worked with was too high for the sand filter to cope with. He does not mention the dilution in which his phage was active. We also found that the slow and the rapid sand filters differed in their behaviour towards phage present in raw water. In rapid sand filters where settlement is short, the reduction was not more than 90 per cent, some strains remaining unaffected, but in the settlement process of slow sand filters where the duration is much longer a reduction of 90 to 99 per cent of phage was always found. The slow sand filter was more destructive to phage than the rapid filter.

CONCLUSIONS

(1) Bacteriophage does not play any part in the purification of raw water in sand filters.

(2) The process of sand filtration has a definite destructive action on bacteriophage present in raw water.

(3) Settled water in slow sand filtration showed 90 to 99 per cent reduction of phage whereas in the settled water of rapid sand filters after the addition of alum we did not find more than 90 per cent reduction. Some of the strains in the latter case remained unaffected.

(4) Slow sand filters showed more destructive action on phage than rapid sand filters. In the former the phage in the majority of cases became completely inactive but in the rapid sand filters reduction was generally 90 to 99 per cent but some strains were also rendered completely avirulent.

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PRELIMINARY OBSERVATIONS ON AN ACID-FAST ORGANISM ISOLATED FROM HUMAN LEPROUS LESIONS

BY

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[Received for publication, February 6, 1931]

INTRODUCTORY

THE micro-organism which is the subject of these observations was originally isolated in a case of human leprosy by my senior colleague, Dr E Mun, who employed the technique described by Shiga (1929). The details of this technique are briefly as follows. Material from a suitable source is crushed in a mortar with 5 per cent H_2SO_4 . The resulting suspension is placed in the incubator for 20 minutes and is then rapidly spun in the centrifuge, the mouth of the centrifuge-tube being closed with a circle of 'Japanese paper' moistened with 5 per cent carbolic acid. The supernatant fluid is poured off, physiological saline is added to the deposit, and the mixture again spun. The sediment from this second spinning is spread with a platinum loop on a 4 per cent glycerine-bouillon-potato medium in Roux tubes, the pH of the medium should be between 6.8 and 7.0.

On looking over my colleague's notes (for this part of the investigation was carried out while the present writer was on leave in England) it appears that, owing to a manifest error in translation, physiological saline containing one-half per cent agar instead of physiological saline alone was used in washing the deposit mentioned above. Whether this made any difference to the subsequent findings it is impossible to say.

The period that elapsed between the date of original explantation and the first sign of visible growth was about eight months, the growth in question is described as being cauliflower-like, grey in colour, about 6 mm in diameter, and raised about 2 mm from the surface of the medium. Smears from this growth revealed acid-fast granular organisms, 'some blue in colour, some

swollen, some long' Subculture on Petroff's medium was successful, a chromogenic, orange-coloured growth resulting after an incubation period of about a week. The organisms from this subculture had the same microscopic appearances as those in the original culture.

Personal observations—Selecting a Petroff subculture in which growth was proceeding vigorously, the present writer attempted to carry the investigation further, and the following is an outline of the results so far obtained.

I CULTURE EXPERIMENTS

A Solid Media—Twelve tubes of Petroff's medium were inoculated, of these, six were incubated aerobically at 37°C, and three under partial anaerobiosis at the same temperature, the remaining three were incubated aerobically at 100m temperature, which at the time of experiment averaged 18.6°C in the twenty-four hours. Partial anaerobiosis was very simply effected—the culture tube having been inoculated, the cotton-wool plug was burnt flush with the mouth of the tube and pushed down for about an inch, a layer of pyrogalllic acid a quarter of an inch deep was then placed on top of the plug, a few drops of freshly-prepared 10 per cent NaOH added, and a second plug quickly inserted, the whole was then rapidly sealed with melted paraffin. In order to arrive at a rough estimate of the degree of anaerobiosis actually attained, the following blank test had previously been performed. Three solutions are made up: (1) Deci-normal NaOH 6 cc, H₂O 100 cc (2) Glucose 6 g, H₂O 100 cc (3) 0.5 per cent aqueous methylene blue 3 cc, H₂O 100 cc. Approximately equal quantities of solutions (1) and (2) are mixed and a few drops of solution (3) added, on boiling, the colour of the methylene blue is discharged and a colourless solution results, the colour is regained in the presence of free oxygen. We carried out this test in a small test-tube, placed it quickly into a large test-tube, and treated the latter by the pyrogalllic acid method described above. By using this two-tube method instead of carrying out the whole process in one large test-tube one avoids to some extent the possible objection that most of the air had been expelled immediately before the pyrogalllic acid treatment and that hence the results are not comparable with those in the main test. A faint blue tint returned to the fluid over a period of several days but we considered that we had obtained a degree of anaerobiosis sufficient for our present purpose. Twelve tubes of nutrient agar and eleven tubes of glycerine agar were also inoculated, divided into three groups, and incubated under conditions similar to those obtaining in the Petroff series.

Results—The cultures were examined daily and by the fifth day growth was sufficiently definite to allow the following notes to be made —

29th December 1930 *Cultures examined*

(a) Petroff, aerobe, 37°C. Three out of 6 tubes show definite yellowish-brown growth, like toasted bread crumbs.

PLATE IV

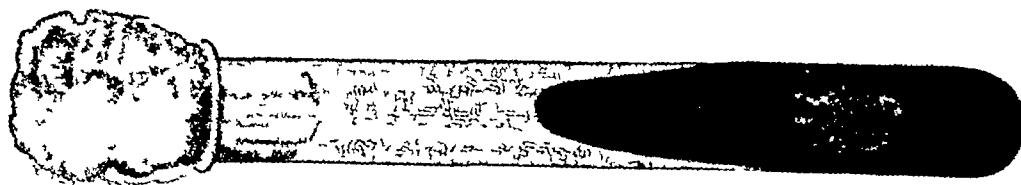


Fig 1—15-day culture on Petroff's medium aerobe 37°C. (The iridescence referred to in the text is demonstrable only on looking along the surface of the medium)

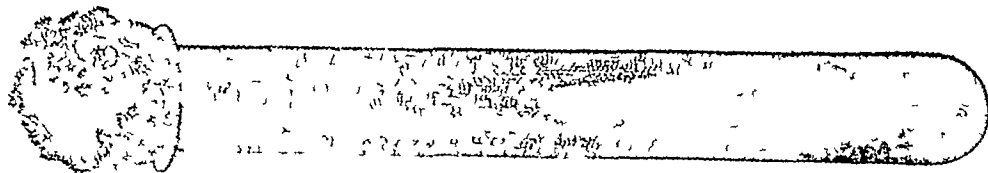


Fig 2—15-day culture on nutrient agar aerobe 37°C

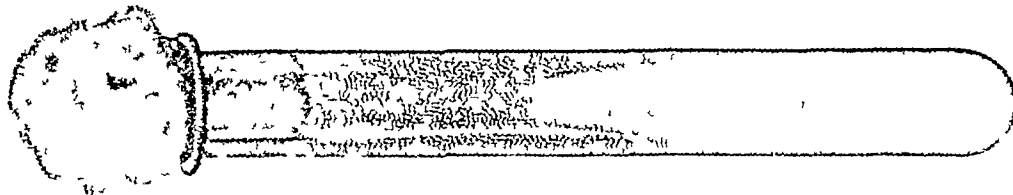


Fig 3—15-day culture on glycerine agar aerobe 37°C

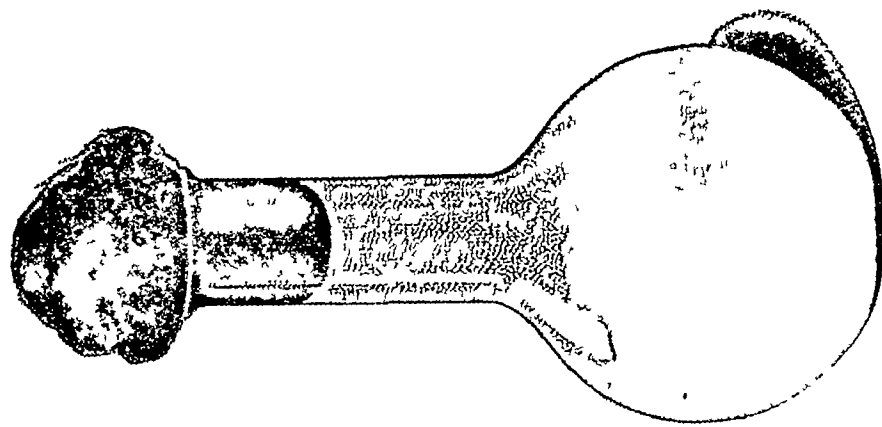


Fig 4—12-day culture on glycerine broth

(b) Petroff, *æ*iobe, room temperature All 3 tubes, slight yellowish-brown growth

(c) Petroff, an*æ*iobe, 37°C Nothing definite

(d) Nutrient agar, *æ*iobe, 37°C All 6 tubes show definite yellowish-white growth

(e) Nutrient agar, *æ*iobe, room temperature All 3 tubes, slight yellowish-white growth

(f) Nutrient agar, an*æ*iobe, 37°C One out of 3 tubes, ? slight growth

(g) Glycerine agar, *æ*iobe, 37°C All 5 tubes, definite yellowish-white to orange growth

(h) Glycerine agar, *æ*iobe, room temperature Two out of 3 tubes, slight orange-yellow growth

(i) Glycerine agar, an*æ*iobe, 37°C One out of 3 tubes, slight yellowish-white growth

By the fifteenth day growth was well established and the following notes were made —

8th January 1931 *Cultures examined*

(a) Petroff, *æ*iobe, 37°C Orange crumb-like growth definite in 3 tubes, doubtful in 2, negative in 1 There is a narrow zone of iridescence on the surface of the culture medium immediately around actively growing cultures (Plate IV, fig 1)

(b) Petroff, *æ*iobe, room temperature Slight orange crumb-like growth with iridescence all 3 tubes

(c) Petroff, an*æ*iobe, 37°C Yellowish growth with iridescence, 1 tube Growth is pale and more confluent than (b)

(d) Nutrient agar, *æ*iobe, 37°C Yellowish slightly confluent growth in all 6 tubes, no iridescence noted (Plate IV, fig 2)

(e) Nutrient agar, *æ*iobe, room temperature Pale yellowish growth, slightly confluent, all 3 tubes No iridescence

(f) Nutrient agar, an*æ*iobe, 37°C Nil

(g) Glycerine agar, *æ*iobe, 37°C Orange-yellow growth more confluent than Petroff, all 5 tubes No iridescence (Plate IV, fig 3)

(h) Glycerine agar, *æ*iobe, room temperature Slight orange-yellow growth all 3 tubes Appears moister than (g) No iridescence

(i) Glycerine agar, an*æ*iobe, 37°C Yellowish-white confluent growth, 1 tube No iridescence

B Liquid Media — (1) Three flasks were taken each containing approximately 100 c c glycerine broth (pH by capillator, 7.3) and surface inoculations were made from the original Petroff subculture Two of the flasks were incubated at 37°C, and one at room temperature, all under fully *æ*iobic conditions (2) Four flasks of the asparagin medium (pH by capillator, 7.2) described by Calmette *et alii* (1926) for the cultivation of B C G, were similarly inoculated, divided into two equal batches, and incubated under

conditions analogous to those obtaining in the case of the glycerine broth cultures

Results—The flasks were examined daily, and by the third day growth was observable in the glycerine broth flasks incubated at 37°C in the form of small greyish translucent pellicles radiating out from the explants on the surface of the medium. These pellicles fused together and formed a continuous film of growth which by the twelfth day completely covered the surface of the medium, and encroached on the walls of the flask. The film of growth now had a wrinkled appearance, creamy in colour with flecks of orange where it impinged on the walls of the flask, there was in addition a granular deposit at the bottom of the flask (Plate IV, fig 4). In the case of the glycerine broth flask simultaneously inoculated and placed in the cool incubator at room temperature, no definite signs of growth were discernible. On the fifteenth day after inoculation this flask was transferred to the 37°C incubator, by the third day thereafter definite growth was visible in the form of greyish translucent pellicles a film formed which, on the expiry of a further period of four days, had reached the walls of the flask.

In the asparagin flasks incubated at 37°C very delicate colourless pellicles, best seen on looking along the surface of the medium, were visible by the third day following inoculation. Growth was much slower here than on the glycerine broth medium and at one point it was thought that it had ceased altogether, but on examining the flasks again on the twenty-first day a complete film of surface growth was detected by the series of ripples which appeared momentarily in it on gently tapping the flask. At present the film is in both instances thin, colourless and delicately wrinkled, thus contrasting with the glycerine broth films, a granular deposit appears to be forming at the bottom of the flasks. In the asparagin flasks incubated at room temperature there are colourless pellicles which seem to be slowly increasing in size.

II STAINING EXPERIMENTS

On the day on which the various media already detailed were inoculated, smears were made on slides from the original Petrioff subculture, stained with cold carbol-fuchsin for 20 minutes, decolorized with 2 per cent acid-alcohol for a few seconds, and counterstained with Manson's borax-methylene blue for 10 seconds. The organism revealed by this method is, in the main, acid-fast, straight or irregularly curved, and occurs both in clumps and singly, many are beaded (showing metachromatic granules) or fragmented. The length varies from 1.5 to 4.0 μ and the breadth from 0.5 to 0.6 μ , an occasional non-acid-fast blue-stained form was noted (Plate V, fig 1). Merely in order to satisfy his sense of curiosity, the writer prepared a further series of slides from the original subculture already mentioned, fixed them, treated them with xylol and then with absolute alcohol (5 minutes each), blotted dry, and stained as before. It was discovered that this procedure results in a loss of

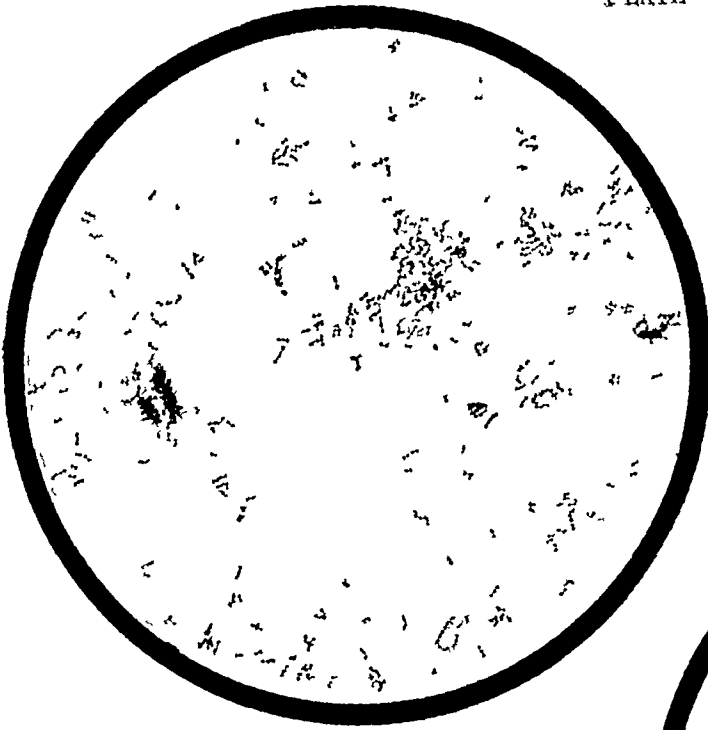


Fig 1—Smear from 15-day culture
on Petroff's medium Ziehl Neelsen's
stain magnification 720 \times

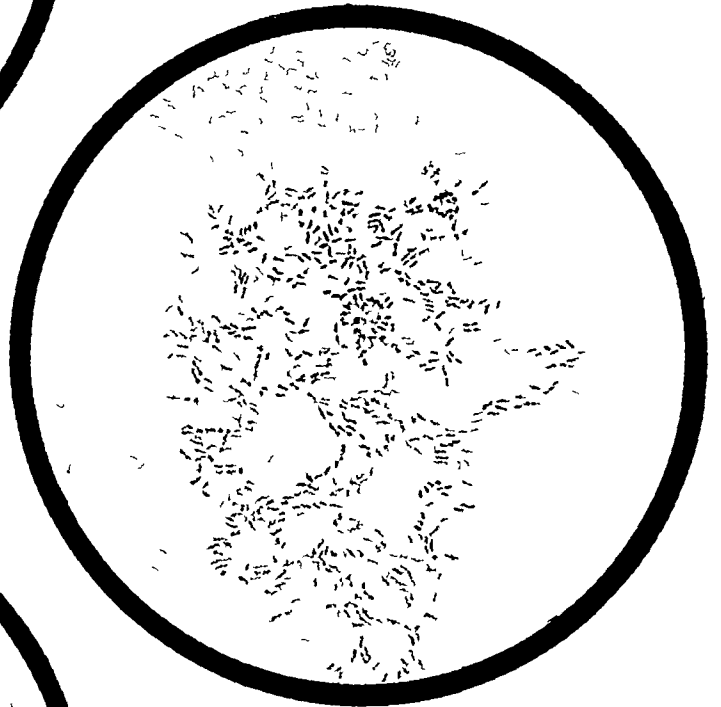


Fig 2—Smear from 15-day culture
on Petroff's medium Treated with
zylol and alcohol absolute followed
by Ziehl Neelsen's stain magnification
720 \times

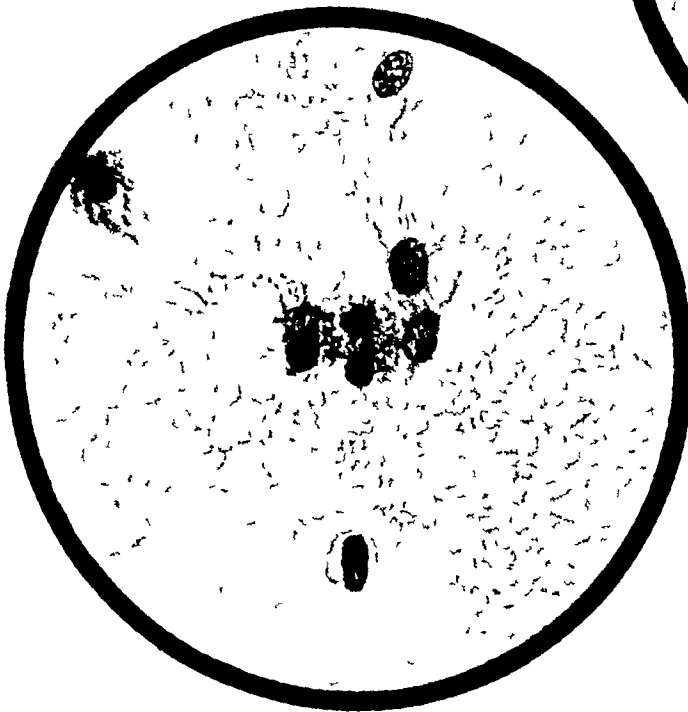


Fig 3—Smear from rat leprous lesion
Treated with zylol and alcohol
absolute followed by Ziehl Neelsen's
stain magnification 720 \times

the acid-fast property in a considerable number of the organisms so that red-stained and blue-stained forms can be seen side by side in nearly every microscopic field (Plate V, fig 2)

I next determined to see whether this change could be effected in organisms from naturally acquired human leprous lesions or in the artificially induced rat disease. Smears were made from suitable lesions from the respective sources, treated with xylol and absolute alcohol as described above and stained in the usual way. In the case of the human organisms no such effect was noted, in the case of the rat organisms a totally different phenomenon was observed—previous treatment with xylol and absolute alcohol appears to render a considerable number of these organisms unstainable so that pale ghost-like bacilli are noted against a background formed by the methylene blue counterstain (Plate V, fig 3). Ghost-like bacilli edged with blue were also noted. Smears from cultures subjected to the action of xylol and absolute alcohol prior to staining are referred to below as treated smears and the following notes show in brief the other observations on staining which have so far been made.

7th January 1931 Glycerine broth cultures, 12th day

Smears made from surface growth

(1) Fixed and stained cold in the usual way

(2) Fixed, treated and stained

(1) Small acid-fast bacillus, appears to be smaller than the organism obtained from the original Petroff subculture, fragmented forms scanty, forms with metachromatic granules and blue-stained forms rare

(2) Treatment does not appear to affect the acid-fast property, very few definite blue forms seen

8th January 1931

Smears from Petroff *aerobe* 37°C, glycerine agar *aerobe* 37°C, nutrient agar *aerobe* 37°C, all cultures 15 days old, two smears from each, one fixed and stained direct as before, the remaining smear from each batch fixed, treated and stained

(1) Petroff *aerobe* 37°C—direct stain. Acid-fast organism similar to that noted in original Petroff subculture, fragmented and beaded forms seen, blue forms in appreciable numbers

(2) Petroff *aerobe* 37°C—treated. As above, but blue forms very numerous

(3) Glycerine agar *aerobe* 37°C—direct stain. As in (1) above, but blue forms very rare

(4) Glycerine agar *aerobe* 37°C—treated. As in (3) above, no apparent increase in blue forms

(5) Nutrient agar α iobe 37°C—direct stain As in (1) above, blue forms appreciable

(6) Nutrient agar α iobe 37°C—treated As in (5) above, no apparent increase in blue forms

9th January 1931

Smears made from Petroff an α iobe 37°C, and from glycerine agar an α iobe 37°C, both cultures 16 days old Glycerine agar an α iobe appears to be moister and less crumbly than the others, Petroff an α iobe is like Petroff α iobe in consistency Two smears from each, one fixed and stained direct as before, the other fixed, treated and stained

(1) Petroff an α iobe 37°C—direct stain Acid-fast organism as before, but numerous pale pink rather swollen forms also seen, blue forms appreciable

(2) Petroff an α iobe 37°C—treated Blue forms more prominent

(3) Glycerine agar an α iobe 37°C—direct stain Blue forms rare, few pinks Very few granular or metachromatic forms

(4) Glycerine agar an α iobe 37°C—treated As in (3) above

17th January 1931

Asparagin culture at 37°C, twenty-second day of incubation, two smears made from surface growth, one fixed and stained direct as before, the other fixed, treated and stained

(1) Asparagin culture—direct stain Small plump acid-fast bacilli relatively scanty, with deeply staining inclusions, appreciable blue forms, acid-fast diplococcoid forms also seen

(2) Asparagin culture—treated As in (1) above

In all the staining observations smears have also been made and stained wet with a modified toluidin blue stain devised and used by my colleague, Dr C McGune, in his mycological studies, in order to see whether branched forms of the organism could be detected, but so far my results have been consistently negative with this stain

III ATTEMPTS TO DEMONSTRATE THE PRESENCE OF BACTERIAL ANTIBODIES

(1) A 14-day glycerine broth culture growing actively at 37°C was filtered under negative pressure through a Doulton's unglazed filter candle (150 \times 38 mm) in a Massen's filtering apparatus Human serum was obtained (a) from advanced untreated cases of nodular leprosy, (b) from non-leprous control Fixed amounts of the filtrate were then added to varying amounts of the sera in order to see whether precipitin formation could be detected The test was done in Dreyer's agglutination tubes and the various constituents were added by means of a Dreyer dropping pipette, the same pipette was used throughout and it was washed out three times with distilled

water, alcohol, and acetone, when changing from one constituent to another. The following is an example of such a test —

Tube No	1	2	3	4	5
N saline (drops)	<i>Nil</i>	5	8	9	10
Serum (drops)	10	5	2	1	<i>Nil</i>
Filtrate (drops)	15	15	15	15	15
Final conc of serum	1 in 25	1 in 50	1 in 125	1 in 250	Control

The tests were read immediately and then placed in the incubator at 37°C for 24 hours, a final reading was then taken. With both groups of sera the results were completely negative.

(2) I next took two flasks of freshly prepared sterile glycerine broth (pH 7.3), each flask containing approximately 100 cc of medium. To flask (A) was added 10 cc of the filtrate noted above, flask (B) was kept as a control, the surface of the medium in each flask was then inoculated with a loopful of a 17-day glycerine agar culture. At the same time six tubes of freshly prepared glycerine agar were taken and to each was added 3 drops of the filtrate, the tubes were inclined to and fro so that the surface of the medium was thoroughly moistened by the filtrate, five tubes of glycerine agar from the same batch, untreated by filtrate, were used as controls. All eleven tubes were inoculated from a 17-day glycerine agar culture and flasks and tubes were placed in the incubator at 37°C. The following notes show the progress of events in this series of observations —

12th January 1931 Cultures examined (3rd day after setting up)

Flask (A) Slight commencing growth. Flask (B), control, doubtful. On the following day definite growth was observable in the latter flask and surface films formed which, by the 7th day after inoculation, had reached the walls of the flasks in both instances. Two smears were made from the surface growth in each flask, one smear from each series was fixed and stained direct as usual, the other fixed, treated and stained.

Flask (A) (Broth plus filtrate)

Smear (1) —Direct stain. Acid-fast bacilli-form organism, fragmentation and metachromatic granules present, occasional blue forms.

Smear (2) —Treated. As in (1) above. No apparent increase in blue forms.

Flask (B) (Broth alone control)

Smear (1) —Direct stain. As in (1) above.

(5) Nutrient agar *aëro*be 37°C—direct stain As in (1) above, blue forms appreciable

(6) Nutrient agar *aëro*be 37°C—treated As in (5) above, no apparent increase in blue forms

9th January 1931

Smears made from Petrioff *anæro*be 37°C, and from glycerine agar *anæro*be 37°C, both cultures 16 days old Glycerine agar *anæro*be appears to be moister and less crumbly than the others, Petrioff *anæro*be is like Petrioff *aëro*be in consistency Two smears from each, one fixed and stained direct as before, the other fixed, treated and stained

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(2) Petrioff *anæro*be 37°C—treated Blue forms more prominent

(3) Glycerine agar *anæro*be 37°C—direct stain Blue forms rare, few pinks Very few granular or metachromatic forms

(4) Glycerine agar *anæro*be 37°C—treated As in (3) above

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Smear (2) —Treated. As in (1) above. No apparent increase in blue forms.

Flask (B) (Broth alone, control)

Smear (1) —Direct stain. As in (1) above.

Smear (2) —Treated As in (1) above, but blue forms appear to be more numerous

(3) Two actively growing 20-day cultures on glycerine agar were scraped off into sterile normal saline containing 0.5 per cent carbolic acid, this carbolic-saline suspension was then ground up as finely as possible in a sterile ground-glass mortar, the operation being carried out inside a sterilized tissue-culture box. The suspension so obtained was then roughly standardized against standard opacity tubes and it was found that it was approximately equal in density to that of a suspension of *Bact. typhosum* containing 630 million organisms per 1 c.c. Serum from a patient suffering from advanced nodular leprosy (untreated) was then obtained and varying amounts were put up with a fixed volume of the bacterial suspension. The method of carrying out the test is similar to that detailed in the precipitin experiments (*vide ante*) except that bacterial suspension instead of bacterial filtrate was added. The final concentrations of the serum were, as before, 1 in 25, 1 in 50, 1 in 125, and 1 in 250, the fifth tube was a control without serum. The tests were put in a water-bath at 55°C for two hours and the first readings were then taken, after a further period of twenty-four hours in the incubator at 37°C, the final readings were taken. At the end of the two-hour period all the tubes including the control showed some precipitation of the suspension with opalescence of the supernatant fluid, after a further period of twenty-four hours at 37°C there was still no evidence of true agglutination.

Summarizing the results of the above small group of experiments (1) We have so far failed to detect in the filtrate from young, actively-growing cultures of the organism any anti-substance which interferes with the subsequent growth of this organism. (2) We have similarly failed to detect in sera from advanced untreated cases of human leprosy any anti-substance or substances which have a demonstrable effect on the organism or of the filtrate therefrom *in vitro*. It should be noted, however, in connexion with the last-mentioned observations that such negative findings do not necessarily rule this organism completely out of court so far as its relationship to human leprosy is concerned. The application of serological *in vitro* methods to the differentiation of the acid-fast bacteria is generally admitted to be unsatisfactory. The agglutination test in particular is of almost no value, firstly because of the supreme difficulty of obtaining a homogeneous suspension of such organisms and, secondly, because of the almost ineradicable tendency of such suspensions to spontaneous clumping—as happened in the present instance.

IV THE EFFECT OF HEAT AND OF ANTISEPTICS ON THE GROWTH OF THE ORGANISM

(a) A loopful of the suspension which had been prepared for the agglutination tests was inoculated on the surface of a glycerine agar slope and incubated at 37°C. Continuous observation over a period of 15 days failed to reveal any growth. It should be noted that there are three possible lethal factors to be considered, (a) the mechanical grinding action to which the

suspension was subjected in preparation, (b) heat, (c) the antiseptic action of the carbolic acid, each of these should be investigated separately

(b) A swab of cotton-wool was wrapped round a short length of copper wire, dipped in 40 per cent formalin, the excess fluid drained off, and the swab suspended over the surface of an actively growing glycerine agar culture for a period of 24 hours so as to expose the culture to the vapour of the formalin, a subculture was then made on fresh glycerine agar. No growth was observed on continuous observation over a period of 15 days

V DOES THE ORGANISM SHOW SURFACE RUNNERS OR DEEP ROOTS IN CULTURE ?

In an attempt to answer this question I applied the method of culture-section described by Acton and McGune (1927). The test-tube containing the culture killed by formalin as described above was broken, the medium with culture *in situ* removed, and freehand sections were cut with a Gillette safety-razor blade. The sections were then stained with weak carbol-fuchsin and examined with a low-power objective, no surface runners or deep roots could be detected

SUMMARY

Preliminary observations are here recorded on an organism isolated once only in twenty-three trials from human leprosy lesions. It belongs to the acid-fast group of bacteria, it grows readily on ordinary laboratory media both at body temperature and at temperatures of about 20°C, cultures are pigmented and their development is most characteristic on media containing glycerine. Growth is most abundant in the presence of free oxygen but can also occur in conditions of partial oxygen deprivation. In young cultures at least there is no evidence of the production of aerial hyphæ, surface runners, or deep roots. Serological investigations designed to establish a connection between this organism and human leprosy have so far yielded negative results and its relationship to *M. lepræ* (Hansen) is at the moment completely undetermined.

My thanks are due to Dr C McGune of the Department of Pathology and Bacteriology for demonstrating the method of culture-section and to Mr J K Mullick, the artist to the Leprosy Research Laboratory, for the illustrations

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POTENCY OF TIME-EXPIRED ANTIVENOMOUS SERUM
STOCKED UNDER ORDINARY CONDITIONS OF
STORAGE AT THE CENTRAL RESEARCH
INSTITUTE, KASAUH

BY

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[Received for publication, February 6, 1931]

ANTIVENOMOUS serum against Cobra and Daboia venoms manufactured at Kasauli is usually held to retain its full potency for one year from the date of manufacture but may be used for another year with 25 per cent increased dosage to make up for the loss of potency during the latter period. After 2 years from the date of manufacture this serum is now considered as time expired and unfit for therapeutic use. Anderson and Cairns (1925) tested some of the above serum stocked under different conditions of storage and found that there was gradual loss of potency on keeping during the first 6—8 months followed by an enhancement higher than that originally possessed by the serum after 12—14 months storage. Nothing is at present definitely known after 14 months storage. It is, however, presumed that like all other antitoxic sera the antivenomous serum undergoes progressive deterioration after that period. The demand for this serum in India is steadily increasing (as would appear from Table I) and although so far all orders have been successfully met it is apprehended that owing to unforeseen events in future the supply possibly might not be proportionate to the demand.

TABLE I

Statement showing the annual output of antivenomous serum in tubes of 40 c c each

1925	1926	1927	1928	1929
2,407	2,667	2,768	3,310	3,404

Whether under such contingencies it would be advisable or not to use the so-called time-expired serum for life saving purpose specially in cases of emergency when the fresh serum is not available in sufficient quantities has to be considered. Fortunately 19 samples of time-expired serum stocked at room temperature were found in a cupboard at Kasauli ranging from $2\frac{1}{2}$ to 9 years old. Physically all these sera were crystal clear with slight deposit of serum proteins at the bottom which when shaken up imparted a uniform hazy appearance to the contents. The samples after sorting out could be grouped as follows according to their age —

1	1 sample	$2\frac{1}{2}$ years old
2	2 samples	3 " "
3	1 sample	$3\frac{1}{2}$ " "
4	1 "	4 " "
5	4 samples	7 " "
6	3 "	$7\frac{1}{2}$ " "
7	1 sample	8 " "
8	4 samples	$8\frac{1}{2}$ " "
9	2 "	9 " "

As the antivenomous serum manufactured at Kasauli is standardized against Cobra venom alone with the minimum capacity of neutralizing 0.5 mg of venom *in vitro* for each c c of serum, we could only compare the neutralizing capacity of time-expired sera in terms of an original minimum standard namely 1 c c of serum equivalent to 0.5 mg of Cobra venom.

The technique employed was the same as is used for standardization of antivenine which is as follows — The dried Cobra venom is accurately weighed to 4 places of decimal and dissolved to the required concentration in normal salt solution. From the stock solution further dilutions are made containing the desired doses of venom in 1 c c of the solvent. One c c of the venom solution of different strengths is then measured into each of a series of test-tubes and to each tube is added 1 c c of time-expired serum. After mixing the contents, the tubes are incubated at 37°C for half an hour. The 2 c c of venom antivenine mixture are then injected into the pectoral muscles of a

series of pigeons weighing 300 grammes each. The reading is taken after 24 hours and the death or survival of the birds over this period noted. With each series of tests a set of controls to show the minimum lethal dose of the test venom was also put up by injecting each of 4 pigeons with 0.2, 0.3, 0.4 and 0.5 mg of Cobra venom respectively. The same sample of venom was used throughout the experiments.

The neutralizing power of the serum was calculated as being equivalent to the largest dose of venom which when mixed with the serum failed to kill, less the minimum lethal dose of the venom as found by the control test. Thus if in a series of tests, the largest dose which failed to kill the pigeon in 24 hours was 0.9 mg of venom and 1 c.c. of serum the minimum lethal dose being 0.4 mg 1 c.c. of serum was taken as capable of neutralizing 0.5 mg of venom.

The following table shows the result of test on the specific neutralizing capacity of time-expired sera —

TABLE II

Serial No	Age of antivenomous serum	Number of samples tested	Neutralizing capacity of 1 c.c serum in milligrammes of cobra venom	Percentage loss of potency during storage	Percentage potency retained after the period of storage
1	2½ years	1	0.5	<i>Nil</i>	100
2	3 "	2	0.2	60	40
3	3½ "	1	0.5	<i>Nil</i>	100
4	4 "	1	0.3	40	60
5	7 "	4	0.5	<i>Nil</i>	100
6	7½ "	3	0.5	<i>Nil</i>	100
7	8 "	1	0.4	20	80
8	8½ "	4	0.28	45	55
9	9 "	2	0.35	30	70

From the above results it would appear that about 50 per cent of the samples retained their potency to the full extent of the original standard long after the present time limit prescribed for their therapeutic use namely a period of 2 years from the date of manufacture. About 50 per cent of the samples were found deteriorated but they still maintained their potency on an average of 50 per cent of the original strength showing that after prolonged storage the deterioration is quantitative only and that the antivenomous serum

like the anti-diphtheritic serum maintains its specificity (MacConkey, 1917), that is to say it is still capable of neutralizing snake venom *in vitro* for a period much longer than what is now considered to be the time limit

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A NOTE ON THE PROBABLE CAUSES OF UNPLEASANT
REACTIONS FOLLOWING PROPHYLACTIC CHOLERA
INOCULATION, WITH SPECIAL REFERENCE
TO CERTAIN AVOIDABLE FACTORS

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[Received for publication, February 6, 1931]

MANY thousand doses of cholera and prophylactic T A B vaccines are manufactured and sent out from Kasauli every year. Although the bulk of the supply is primarily meant for the Army, a considerable quantity of these vaccines is also used by the civil population of British India and Indian States. The demand varies directly with the number and frequency of epidemics in each year. Every step in the manufacture of these vaccines is controlled by an experienced bacteriologist with the help of about half a dozen trained laboratory assistants working directly under his supervision. The finished vaccines in bulk are stocked in concentrated suspensions and have to be diluted, bottled and tested for sterility before sending them out to points of distribution. This final dilution and bottling in various sizes of ampoules is entirely in the hands of responsible officers. In spite of these stringent precautionary measures it is not unusual to get occasional complaints from medical officers engaged in preventive work, that particular capsules of vaccines produced severe local and general reactions after injection. Such complaints in the past have more often been received in case of cholera than in case of T A B vaccine, particularly from civil practitioners who during epidemics generally use a single immunizing dose of 1 c c per individual instead of an

initial dose of $\frac{1}{2}$ c c followed by 1 c c after ten days' interval. This excessive dosage by itself may produce an aggravated reaction in a person who has not been prepared for a higher dose by a previous injection of $\frac{1}{2}$ c c of vaccine, but such a possibility, however, does not hold good when only a part of the brew is inoculated while no complaint is made against the major portion of the same brew which was also used in 1 c c doses.

Apart from the fact that the vaccine itself may be at fault, there might be other factors at work at the points of distribution which may partly or wholly contribute towards the severity of reaction after inoculation, e.g., non-observance of strict asepsis, use of blunt needles for injection, the latter mainly acting by producing mechanical damage to adjacent parts, etc. These possibilities are matters of mere speculation and cannot be verified or substantiated at the source of manufacture. Consequently, it devolved on us to find out if, at any stage in the manufacture of vaccines, something is happening which can definitely be proved to be the cause of severe reaction after injections. The following possibilities were investigated —

- (1) Impure and contaminated vaccine
- (2) Excessive dosage due to errors in standardization
- (3) Excess of carbolic acid used as preservative
- (4) Excess of foreign proteins (other than bacterial proteins) such as peptones, proteoses and other nutrient materials, together with certain products of bacterial metabolism

In the following paragraphs we propose to discuss how far each of these possibilities may be held responsible for the unpleasant reaction produced —

1 *Impure and contaminated vaccine*

All brews and samples, supposed to have produced unpleasant reactions on injection, were examined culturally both by the aerobic and anaerobic methods and were found free from contamination. They were also examined microscopically by appropriate staining and showed nothing but the organisms which had been used originally.

2 *Excessive dosage due to errors in standardization*

All cholera vaccines are first killed and then standardized by opacity method (Brown, 1919) immediately after preparation. As they undergo progressive autolysis on storage (Harvey, Iyengar and Christophers, 1921) even in isotonic saline, bacterial strengths of such samples, as were unfavourably reported upon, could not be definitely ascertained and compared with the original strength of 8,000 millions of vibrios per 1 c c of the vaccine. It has, however, been frequently found by experimental observations that 1 c c of fresh cholera vaccine of the strength of 65,000 millions per c c does not cause any appreciable local reaction or necrosis when injected subcutaneously into guinea-pigs.

PLATE VI



Fig 1—Appearance 24 hours after subcutaneous injection of cholera vaccine containing the minimum amount of nutrient material and foreign proteins No necrosis



Fig 2—Appearance 24 hours after subcutaneous injection of cholera vaccine containing an excess of foreign proteins and soluble products of bacterial metabolism Necrosis evident

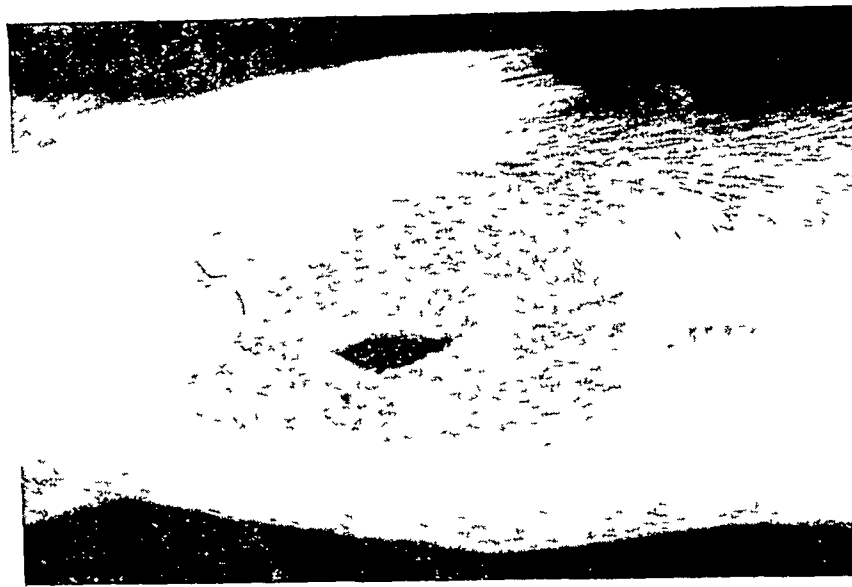


Fig 3—Appearance 72 hours after subcutaneous injection of cholera vaccine containing an excess of foreign proteins and soluble products of bacterial metabolism Necrosis evident

3 *Excessive quantity of carbolic acid used as preservative*

Considering that all stocks are diluted in bulk of not less than 4,000 c c at a time, and the requisite quantity of phenol is added at this stage, any error in calculation and measuring of the latter could not possibly be more than a fraction of 1 c c for every 4,000 c c of the finished vaccine. Supposing for the sake of argument, however, it is assumed that one full c c was added in excess, this must have been diluted 4,000 times when 1 c c of the vaccine was given as immunizing dose. Such infinitesimal quantity of excess of phenol could scarcely be expected to give rise to severe reactions. Apart from this theoretical consideration, we actually prepared a vaccine of standard strength with 1 per cent phenol as preservative. This vaccine injected subcutaneously in 1 c c doses into 3 human volunteers and 3 guinea-pigs produced no more reactions than the vaccine to which 0.5 per cent phenol had been added. Severe reactions after cholera inoculation therefore could not have been due to slight excess of carbolic acid content.

4 *Excess of foreign proteins (other than the bacterial protein) such as peptones and proteoses and other nutrient materials together with certain products of bacterial metabolism*

In order to understand how foreign proteins and soluble products of bacterial metabolism can be present in cholera vaccine, it is necessary to describe in detail a few steps in the mass production of this vaccine as practised in Kasauli and presumably also in other laboratories in India.

A twenty-four hours' broth culture of cholera vibrio is planted on rolled cultures of trypticized casein agar in 2 pint whisky bottles, each bottle receiving about 4-6 c c of the broth culture. The inoculum is spread over the agar surface simply by rolling the bottles in hand. These bottles are incubated at 37°C for 24 hours, and the resulting growth of each bottle is washed with about 20-25 c c, of normal salt solution, and decanted into a sterile test-tube of 6 inches \times 1 inch size. These washings contain not only live cholera vibrios but also a certain amount of nutrient material of the nature of peptones and proteoses besides soluble products of bacterial metabolism which were present with the initial broth inoculum squirted into each bottle. The washings in test-tubes are, however, allowed to stand for 48 hours to enable the suspended bacteria to be deposited. The supernatant fluid is then decanted off and the bacterial deposit retained for preparing the vaccine. The supernatant fluid rejected at this stage usually carries away most of the nutrient materials and soluble toxins. If on account of carelessness this step is not properly carried out, an excess of the latter may get into the vaccine and give rise to unpleasant reaction. In order to verify this possibility saline washings of a single brew were divided into two parts —

(1) Supernatant fluid of one half was completely decanted off and a vaccine of the standard strength was prepared from the bacterial deposit alone

(2) In case of the other half, supernatant fluid was only partially decanted off and a vaccine of the same strength was prepared from the mixture of supernatant fluid and bacterial deposit

The above vaccines were injected subcutaneously in 1 c c doses in human volunteers and guinea-pigs. The result has been summarized in the following table —

	Number of volunteers injected	Dosage Each volunteer	Result	Number of guinea pigs injected	Result
Vaccine with excess of supernatant fluid	6	1 c c	3 severe local reactions, with fever and headache, they were unable to attend their ordinary duties for 48 hours 3 moderate reactions only	2	Redness, oedema and local necrosis after 24 hours. Scabs formed at the site after 48 hours to 72 hours
Vaccine practically free from supernatant fluid	6	1 c c	5 moderate reactions only, capable of attending light duties 1 moderately severe reaction	2	Practically no reaction except slight redness for 24 hours after which it cleared off

The comparative effects of two vaccines are also obvious from the photographs of 2 guinea-pigs each of which received 1 c c dose subcutaneously

SUMMARY

(1) Slight excess of phenol in cholera vaccine over the normal 0.5 per cent used as preservative, does not produce any appreciable local reaction when injected subcutaneously in man

(2) Cholera vaccines 8 times as strong in bacterial content as used for preventive inoculation in man, have no severe local and general effects when injected subcutaneously into guinea-pigs

(3) Cholera vaccines of standard bacterial strength, with an excess of foreign proteins—the nature of peptones, proteoses and amino acids, etc,—and soluble products of bacterial metabolism, are responsible for severe local and general reactions after injection both in men and experimental animals

(4) Cholera vaccine prepared from the bacterial deposit and containing the minimum possible amount of nutrient material would, in our opinion, reduce to a very considerable degree untoward reactions following prophylactic inoculation

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ABSENCE OF MALARIA IN THE SALT-WATER LAKE BASIN *

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IN the district of 24-Periganas in Lower Bengal and to the east of Calcutta is an extensive inland saline water basin locally known as the Salt-water Lake. This basin which is ten miles long and over three miles in width is embanked all around and traversed by numerous ridges and embanked footpaths. The deeper portions of the basin hold in saline water throughout the year and these consist of numerous shallow tanks or reservoirs which are worked as fisheries. There are many villages and hamlets distributed over the basin inhabited by the people that work the fisheries in the Salt-water Lake.

During the rainy season, the level of water in the Salt-water Lake Basin rises to such an extent as to cover nearly the entire area, while during the off season, a large part of this shallow basin is dry. During the wet season, the villages situated in the middle of the basin are mere islands many of which are inaccessible except by boats and canoes. In some of these villages, the water rises so high as to cover the entire ground leaving only the plinths of houses above water. One has frequently either to wade through water or to use a canoe even to go from one house to another within the village. Such a condition is not an unusual one during the rainy months. One frequently observes children swimming across the water in order to go to a neighbouring house and they are so much accustomed to such a condition that they do not seem to feel that they do anything unusual.

The flora of the Salt-water Lake Basin comprises several typical saline plants and some characteristic mangrove vegetation. Halophytes like *Suaeda maritima* Dum., and *Tamarix gallica* Linn., and mangrove plants like *Acanthus ilicifolius* Linn., *Erocaria agalocha* Linn., *Avicanna officinalis* Linn., are common in this area. Other mangrove vegetation such as *Sonneratia*, *Ceriops*,

* Read before the Indian Science Congress, Nagpur, January, 1931

Kandela, *Ægiceras* and *Bruguiera* also occur though less commonly *Enteromorpha*, a characteristic salt-water alga, is very common in the water of the lake and in ponds and ditches, *Oscillatoria*, a blue-green alga which is also very common, frequently forms dense felted masses which float on the surface of the water. The Salt-water Lake Basin with its fishery reservoirs, its saline flora and the villages subject to flooding during the rainy season is in many ways an interesting formation.

COMMONLY BELIEVED TO BE INTENSELY MALARIOUS

The common lay person would put this area down as an intensely malarious one. In fact this lake has had the unenviable reputation of being a dangerously malarious tract and many persons have ascribed the unhealthiness of the City of Calcutta to the proximity of the Salt-water Lake. In the earlier official records there are numerous references to the supposed unhealthiness of the Salt-water Lake. As early as 1706, Capt Hamilton is reported to have described the influence of the Salt-water Lake on the health of Calcutta in the following terms: 'Mr Charnock could not have chosen a more unhealthy situation on all the line of river, for three miles to the eastward is a salt-water lake, which overflows in September and October and prodigious numbers of fish resort there, but in November and December, when the floods are dissipated, those fishes are left to die, and with their putrefaction affect the air with thick stinking vapours, which the north-east winds bring with them to Fort William, so that a great yearly mortality is caused by them'*. Even medical men have expressed views entirely similar to the one above quoted. Dr Duncan Stewart in 1836 expressed his opinion that the unhealthiness of the eastern portions of Calcutta was due to the proximity of the Salt-water Lake. He had stated that the eastern part of Calcutta has 'always been considered unhealthy and could scarcely indeed be otherwise situated as it is upon the marshy edge of the Lake and surrounded by dense low jungle. The natives dwelling there are subject to constant low fevers, spleen, dropsy and disease, to new-comers, a residence of even a few days is almost sure to be fatal'†. Dr Strong, Surgeon of the 24-Perganas, wrote as follows in 1837: 'I have visited many of the villages bordering on the Lake and find the inhabitants in point of appearance and health to correspond very much with the state of those people that inhabit malarial and marshy lands'‡.

The lay public as well as the medical profession seemed so fully convinced of the presumed unhealthiness of the Salt-water Lake and of its injurious influence on the health of Calcutta that efforts were made from time to time both by the Government and by the public to reclaim the lake. In 1830, Lord Bentinck, the then Governor-General, strongly recommended that the Salt-water Lake should, on sanitary grounds, be embanked and drained with

* Selections from the records of the Government of Bengal, p 4

† Ditto do do p 25

‡ Ditto do do pp 4-5

a view to mitigate the unhealthiness of Calcutta. The Court of Directors approved of this proposal, but were unable to provide adequate funds at the time. Latterly the matter was taken up in earnest by the public and in 1865, a proposal was made to Government for the promotion of a company to undertake the reclamation of the Salt-water Lake (Inglis, 1909, p 562). This proposal was submitted to a commission appointed by Government to examine the matter in detail. After an investigation, the commission reported that 'the weight of evidence establishes the fact that the existence of the tract in its present state is most injurious to the health of Calcutta'. In recommending the adoption of the scheme, the commission expressed its opinion that if the scheme is carried out, it would 'substitute a cultivated drained area for the present pestilential swamp and forest' and as a result would greatly improve the health of Calcutta (Inglis, 1909, p 567). The proposal for the promotion of the company fell through owing to some technical objections regarding grant of land and certain rights, but although the scheme was dropped, the Government still viewed the question with much favour. In 1880, the Government of Bengal passed a resolution to say that the 'reclamation of the Salt-water Lake is a project which the growing prevalence of fever in Calcutta makes it desirable to see again brought forward'*

A MALARIA-FREE AREA

In contrast to the beliefs mentioned above, the results of the present extensive survey of the Salt-water Lake Basin show that it is a healthy area almost entirely free from malaria. It seems fortunate therefore that all the proposals put up from time to time for the reclamation of the Lake had fallen through and thus avoided waste of some enormous expenditure on these projects. When we find that the Lake area is quite healthy, it is inconceivable that it should have anything but a beneficial influence on the health of Calcutta.

A detailed malaria survey of the entire Salt-water Lake Basin has recently been concluded. In this connection, 83 villages situated in the Basin were studied and about 6,000 children, of 12 years and under, examined for the estimation of the spleen rate of the villages. The gross spleen rate for the entire area works to 0.3 per cent and this shows that the Salt-water Lake area is remarkably free from malaria. The details of the results of this survey are tabulated below —

Number of children examined	Number with enlarged spleen	Spleen rate Per cent	DEGREE OF SPLENIC ENLARGEMENT					
			F1	F2	F3	F4	U	BU
5,888	17	0.3	5	7	4	1		.

* Selections from the records of the Government of Bengal, p 130

Out of the 83 villages surveyed here, 71 of them had a spleen rate of zero. Of the remaining 12 villages, eight of them had spleen rates of 2 per cent and under. Only four villages out of the 83 surveyed had spleen rates above 2 per cent, the highest spleen recorded in the area being 8 per cent. The respective spleen rates of the different villages surveyed are marked on the sketch map of the basin (see Map opposite). Details of the result of examination of children in these villages are furnished in the Appendix. The gross spleen rate being so low as 0.3 per cent among a scattered population in a rural area where no sanitary measures are carried out, we may take it that malaria is practically absent in the Salt-water Lake Basin which appears to have a natural freedom from malaria.

THE ANOPHELINE FAUNA

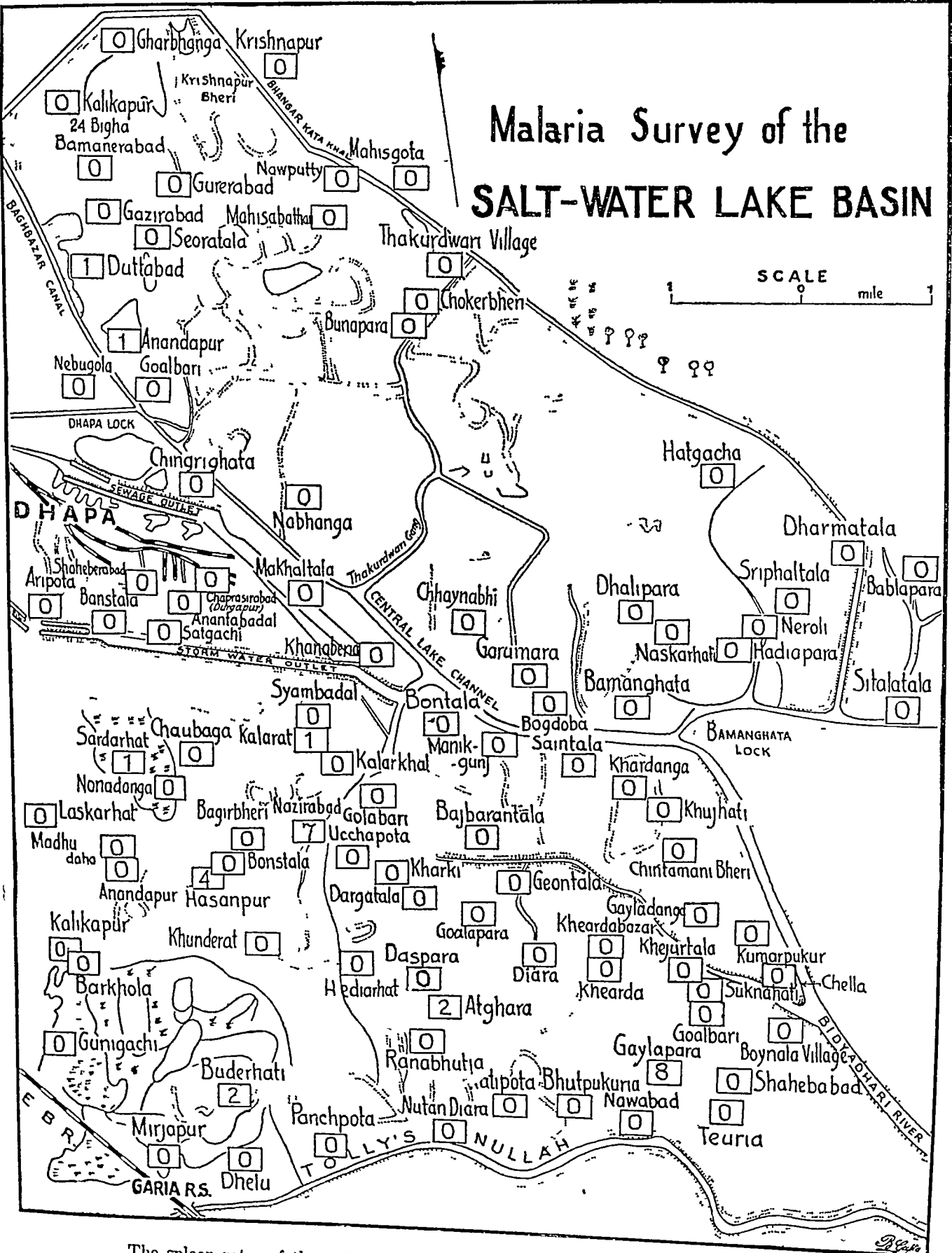
The incidence of anopheline mosquitoes in the Basin has been studied both from adults caught in the villages and from larvæ collected from breeding places. The following seven species occur here, namely, *Anopheles subpictus* Grassi (rossi Giles), *A. vagus* Don, *A. hyrcanus* var. *nigerrimus* Giles, *A. barbrostus* Wulp, *A. fuliginosus* Giles, *A. pseudojamesi* Stål and Ch. and *A. varuna* Iyengar. Judged from the numerical prevalence of adult mosquitoes as well as of larvæ, *Anopheles subpictus* is undoubtedly the predominant species in the area. This species comprises more than 90 per cent of the total catch and in several villages it was found that the anopheline population consisted almost entirely of this one species. It breeds in very large numbers in saline and blackish water, specially those with salt-water algæ like *Enteromorpha* and *Oscillatoria*. In many places, a single dip of the collecting pan brought up over a hundred larvæ and pupæ consisting of this species and it seems evident that this type of breeding place is very favourable for *A. subpictus*. With such heavy breeding almost everywhere, it is not surprising that we should have a very heavy incidence of adult *A. subpictus* in these villages. The walls in cattle sheds and dwelling houses are frequently thickly covered over with *A. subpictus* mosquitoes.

The other six species of *Anopheles* mentioned previously occur very sparsely in this area and they are evidently not of much importance here. While the high prevalence of *A. subpictus* is explainable by the abundance of suitable breeding places such as the saline and blackish water collections, the sparseness of the other species of *Anopheles* is due to lack of breeding places suitable for those species. Fresh water collections in which the latter ordinarily breed are entirely wanting in the area.

Anopheles subpictus AND MALARIA

The absence of malaria in the villages situated in the Salt-water Lake Basin is attributable to the sparseness of carrier anophelines. At the same time, the enormous prevalence of *Anopheles subpictus* in every one of these

Malaria Survey of the SALT-WATER LAKE BASIN



The spleen rates of the individual villages are indicated by figures in small squares

villages which have little or no malaria seems to prove conclusively that this mosquito is a non-carrier in nature

Literature on the subject of the susceptibility of *Anopheles subpictus* to infection with malaria parasites is not entirely unanimous. While a large number of the workers on the question have obtained consistently negative results, several of them have recorded the finding of *A. subpictus* infected experimentally and in some instances even in nature. Some of these findings of infected *A. subpictus* really referred to *A. ludlowi* as was pointed out by Bentley (1911) and later by Covell (1927, pp. 80-81). In fact, some twenty years ago several of the workers believed that the two species *A. ludlowi* and *A. subpictus* were identical and did not therefore maintain separate records for them (Strong, 1910). The earlier records of *A. subpictus* infectivity, in those localities in which *A. ludlowi* also occurs, are not entirely reliable owing to the possibility of *A. ludlowi* being mistaken for *A. subpictus* or being mixed up with it.*

In the absence of convincing evidence to show that *Anopheles subpictus* plays any appreciable part in the epidemiology of malaria, various explanations have been put forward from time to time to correlate the finding of *A. subpictus* susceptible to infection under experimental conditions with its apparent innocuousness in nature. It has been argued, for instance, that although *A. subpictus* is susceptible to infection, the development of the parasite goes only as far as the early oocyst stage and as the infection does not reach the final infective stage of sporozoite in the salivary glands, the mosquito cannot act as an efficient transmitter. This view is not entirely correct as instances in which *A. subpictus* was found with sporozoites in the salivary glands have been recorded (Gill, 1925; Soesilo, 1928).

Another view which has already been controverted is that of Vogel (1910) who thought that the infectivity of *A. rossii* varied with the degree of salinity of the water in which it bred. He tried to show that *A. rossii* mosquitoes bred out of saline water were more susceptible to infection than those bred

* Even in the case of later records from areas where these two species occur side by side, this possibility is not entirely excluded owing to the fact that it is frequently very difficult to distinguish old specimens of *A. ludlowi* much denuded of their leg scales from specimens of *A. subpictus* as they are so very similar to the latter. The writer has experienced much difficulty in the identification of old specimens of *A. ludlowi* in which the speckling of the legs is not clearly noticeable. In many of these cases, some help may be had from the nature of the black spotting at the base of the costa. The basal black area on costa of *A. ludlowi* is a comparatively long one and this is a fairly constant feature which can be used as an additional aid in distinguishing this species from *A. subpictus* in which the black area at base of the costa is not generally so extensive. It must, however, be noted that the absence of extensive black scaling on base of costa in *Anopheles subpictus* is not always a constant feature, as it has been observed by the writer that specimens of *A. subpictus* bred from salt-water frequently have a longer black spot on the base of the costa similar to that of *A. ludlowi*.

out of water with a lower saline concentration. Vogel's observations were subject to two fallacies, firstly it has been doubted if he was actually working with *A. rossii* since many workers think that the species he was experimenting with was *A. ludlowi* and not *A. subpictus*. Secondly, his observations on the relation of salinity to the infectivity of the mosquitoes were vitiated by the fact that the patients, on whom these mosquitoes were fed, were at the same time being treated with large doses of quinine. Bentley (1911) has shown that the apparent correlation which Vogel made out between the lowering in the degree of infectivity of the mosquitoes under experiment and the lowering of the salinity of the water could, from Vogel's own figures, be clearly shown to be the result of reduced infectivity of the patients as the result of medication with quinine.

Gill (1925) thinks that *A. subpictus*, although susceptible to infection, is not a good carrier in nature in Punjab, as he considers that this species is susceptible to infection with only *Plasmodium vivax* and not with *P. falciparum* and that the time of prevalence of *A. subpictus* does not correspond with the time of prevalence of *P. vivax* infection. On the other hand, we have the findings of Soesilo (1928) who reports heavy susceptibility of *A. subpictus* equally well to *P. vivax* infection as to *P. falciparum* infection. Soesilo thinks that *Anopheles subpictus*, although very susceptible to infection, does not play any part in the epidemiology of malaria, because, as he thinks, this species is a zoophile and does not ordinarily attack man.

Let us now consider the conditions that prevail in the Salt-water Lake Basin. In this area, malaria is practically non-existent and we have a very heavy incidence of *A. subpictus* throughout the year. It has been presumed that *A. subpictus* bred from salt-water is susceptible to infection, but here, all the *A. subpictus* that occur are from salt-water and yet the area is free from malaria. There could thus be no support to the view that *A. subpictus* bred out of salt-water are malaria transmitters and that those bred out of fresh water are not. Another view is that *A. subpictus* is capable of transmitting only tertian malaria and that its apparent innocuousness may often be due to tertian infection being scanty or absent among the population at the time of the maximum prevalence of this anopheline. While this view has not yet found general acceptance, it certainly does not hold good in the Salt-water Lake Basin, as in the areas surrounding the Basin, tertian infection is by no means uncommon. Soesilo thinks that the reason for *A. subpictus* not acting as an efficient transmitter of malaria in nature is because it is a zoophile and would not ordinarily attack man. In the Salt-water Lake Basin, the population consists almost entirely of fishermen who generally do not maintain many cattle. The land being subject to much flooding, very few cattle, if any, stay in the villages in the central part of the Basin. When one finds such heavy breeding of *A. subpictus* and a high incidence of adult mosquitoes in an area with so few domestic animals, it goes without saying that *A. subpictus* is a homophile, at any rate so far as the present area is concerned. The absence

of malaria here is certainly not due to any dislike of *A. subpictus* for human blood

When one considers the different aspects of the question, there does not seem to be any more acceptable explanation than that *Anopheles subpictus* is a species which is extremely resistant to infection with malaria parasites under natural conditions. It would be relevant to quote the conclusions of Bentley (1911) whose findings over twenty years ago are so much to the point here —

'1 That *N. rossii* is naturally refractory to malarial infection

'2 But that this immunity may sometimes be broken down, notably under conditions inseparable from feeding experiments conducted with mosquitoes in captivity

'3 That there are strong reasons for assuming that *N. rossii* plays no part in the spread of malaria in India'

The present field studies in the Salt-water Lake Basin confirm the findings of Bentley and show that, under natural conditions, this species is innocuous as regards malaria transmission in Bengal

ABSENCE OF *Anopheles ludlowi* IN THE SALT-WATER LAKE

One interesting point in connection with this area is the total absence of *Anopheles ludlowi*, the well known carrier of malaria in spite of conditions here being apparently very favourable for this species to breed. The breeding places here are saline and have much the same saline flora as the typical *ludlowi* breeding places on other parts of Bengal, and so far as can be judged, we can find no essential differences between the fishponds of the coastal region of the Dutch East Indies in which *A. ludlowi* breeds profusely and the ponds in the Salt-water Lake Basin. *Anopheles ludlowi* is abundantly common in the district of Khulna about fifty miles to the east of the Salt-water Lake. It also occurs at Port Canning, about 20 miles south-east, at Takli 30 miles to the east and at Budge-Budge 18 miles to the west of this area. While *A. ludlowi* occurs in so many places in Lower Bengal, it is difficult to explain its absence in the Salt-water Lake. But undoubtedly the present situation is very fortunate as, in all probability, the healthy area, as we find the Salt-water Lake now, would have been intensely malarious if *Anopheles ludlowi* was breeding in the salt and brackish waters where we now have the harmless *Anopheles subpictus*.

The writer desires to acknowledge his indebtedness to Dr Amritlal Sarma Chowdhury, Sub-Assistant Surgeon, Bengal Public Health Department, for his invaluable help in connection with this and other surveys

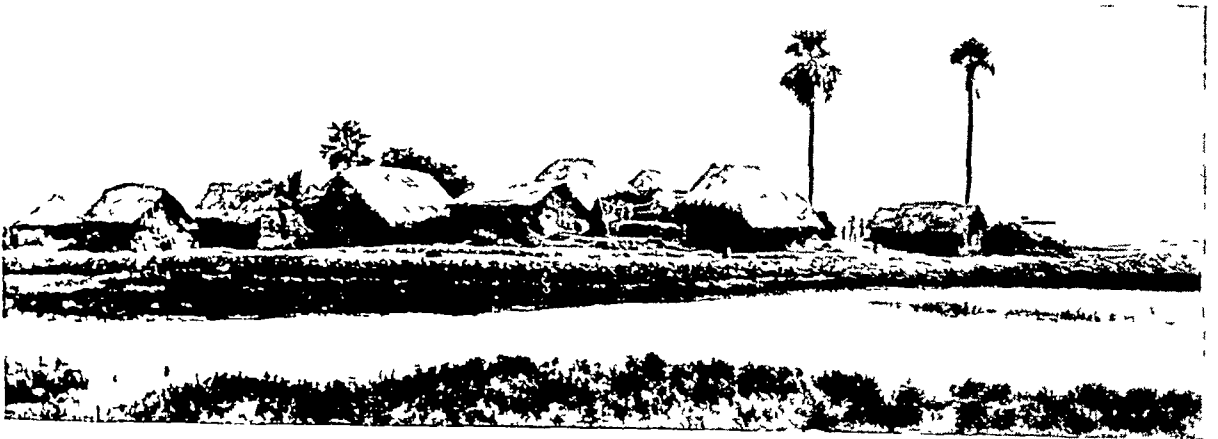
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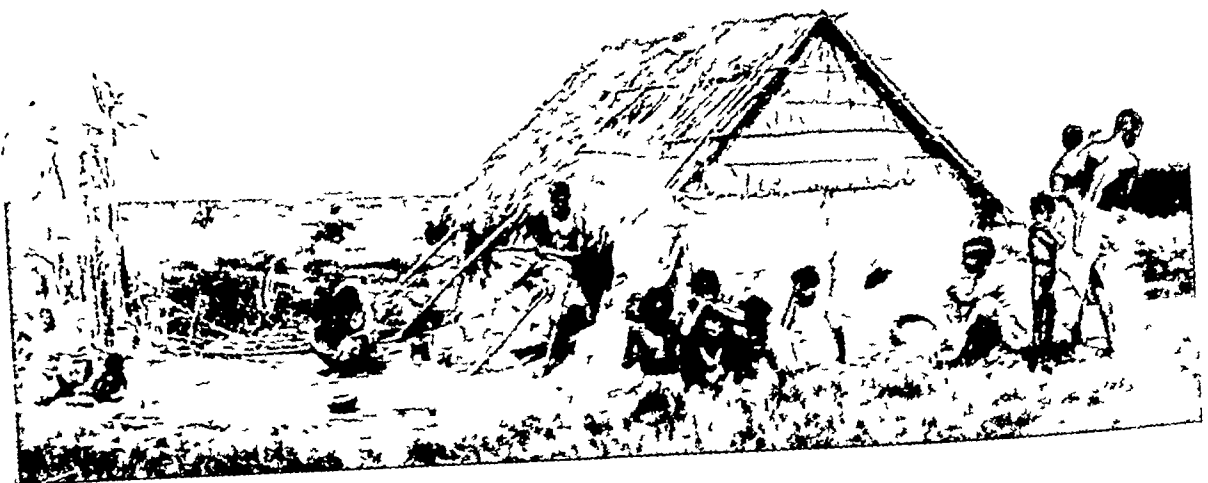
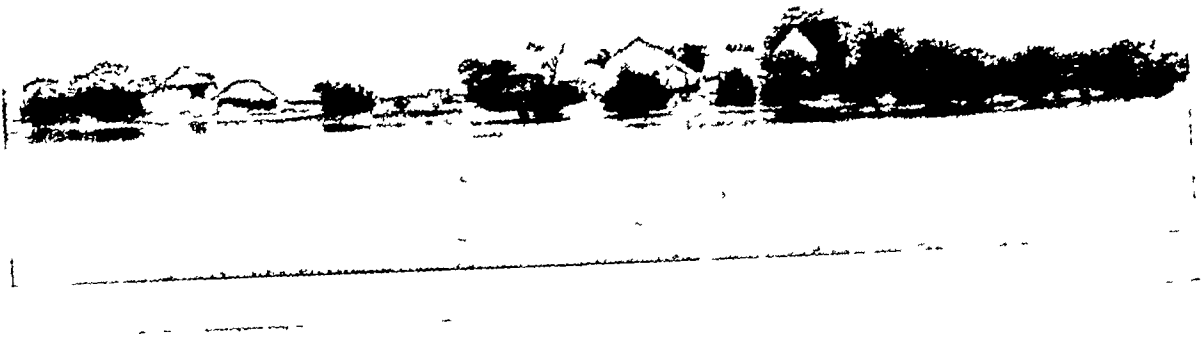
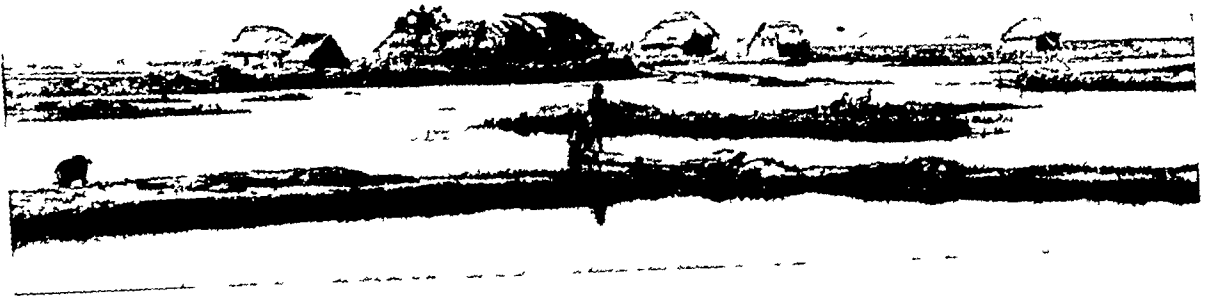
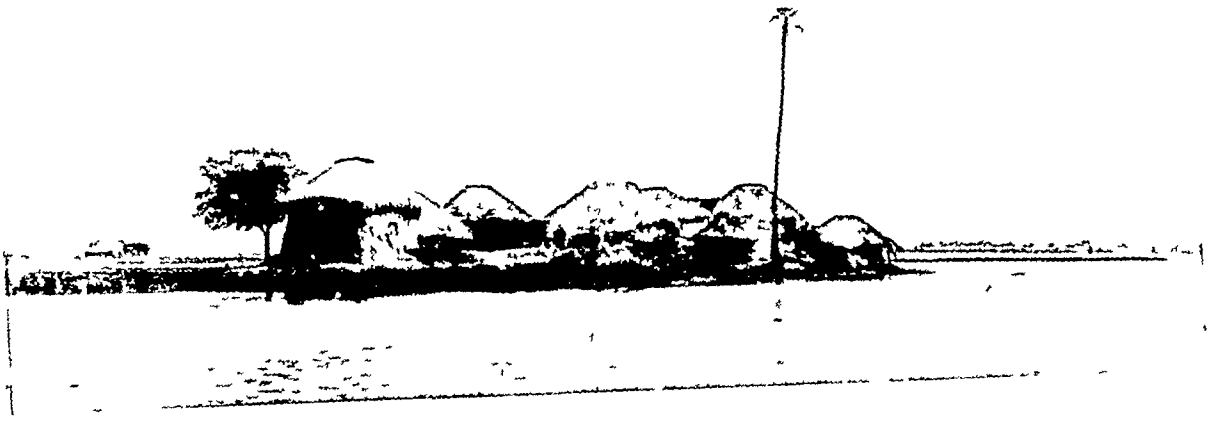
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EXPLANATION OF PLATE VII

Photographs of villages in the Salt-water Lake Basin The villages are situated on raised land and the individual houses have elevated plinths Note the extent of water lying on the land





EXPLANATION OF PLATE VIII

The three photographs on top are of three 'island' villages in the middle of the Salt-water Lake Basin

Bottom A fisherman's hut in a Salt-water Lake village The people here are perfectly healthy The incidence of malaria in these villages is practically nothing

APPENDIX

Malaria survey of the Salt-water Lake Basin

Serial No	Village	Number of children examined	Children with enlarged spleen	Spleen rate Per cent	CLASSIFICATION OF SPLEEN					
					F1	F2	F3	F4	U	BU
1	Buderhat	48	1	2 1	1					
2	Atghara	47	1	2 1		1				
3	Daspara	61	0	0 0						
4	Hedarat	17	0	0 0						
5	Hushanpur	56	2	3 6		1	1			
6	P a i k p a r a, Bagir Bheri	28	2	7 1		1	1			
7	Bastala, Anandapur	68	0	0 0						
8	Madhudaka	42	0	0 0						
9	Chowbagha	249	1	1 4		1				
10	Sadarhat	139	2	1 4			1	1		
11	Khunderat	59	0	0 0						
12	Banstala	105	0	0 0						
13	Sapgachhi	39	0	0 0						
14	Aripota, Dhelenda	123	0	0 0						
15	Saheerabad	18	0	0 0						
16	Chaprasherabad	68	0	0 0						
17	Anantabada	37	0	0 0						
18	Bamanghata	126	0	0 0						
19	Teoria	102	0	0 0						
20	Saheerabad	69	0	0 0						
21	Moulihat	70	0	0 0						
22	Kalarat	87	1	1 1		1				
23	Kalarkhal	51	0	0 0						
24	Uchhapota	62	0	0 0						
25	Nazirabad	28	2	7 1	2					
26	Golabat	99	0	0 0						
27	Kharki	86	0	0 0						
28	Durgabat	45	0	0 0						

APPENDIX—contd

Serial No	Village	Number of children examined	Children with enlarged spleen	Spleen rate Per cent	CLASSIFICATION OF SPLEEN					
					F1	F2	F3	F4	U	BU
29	Deorah	229	1	0.4			1			
30	Nuabad	193	0	0.0						
31	Mukundapur Jagadipota	12	0	0.0						
32	Gharbhanga	24	0	0.0						
33	Duttabad	70	1	1.4		1				
34	Anandapur	68	0	0.0						
35	Kalikapur, 24 Bigha	14	0	0.0						
36	Gazrabad Bamanerabad	26	0	0.0						
37	Gurerabad	30	0	0.0						
38	Seoratola	54	0	0.0						
39	Golabari (Nebugola)	55	0	0.0						
40	Nabhangra	67	0	0.0						
41	Chingrihata	45	0	0.0						
42	Mahishabathan	172	0	0.0						
43	Nawpatti	159	0	0.0						
44	Bajbarantola	121	0	0.0						
45	Hatgachha	251	1	0.4		1				
46	Kochpur, Paschimpara, Hatgachha	293	0	0.0						
47	Dhulapara	71	0	0.0						
48	Dharamtola	71	0	0.0						
49	Baintola	39	0	0.0						
50	Khanabaria	110	0	0.0						
51	Bablapara (Ghuskhali)	42	0	0.0						
52	Thakurdwari	100	0	0.0						
53	Hadiapukur, Naskarhati	47	0	0.0						
54	Neruli (Nerarmath)	17	0	0.0						
55	Sripaltola	38	0	0.0						

APPENDIX—concl'd

Serial No	Village	Number of children examined	Children with enlarged spleen	Spleen rate Per cent	CLASSIFICATION OF SPLEEN					
					F1	F2	F3	F4	U	BU
56	Chhay nabhi	51	0	0 0						
57	Makhatola	19	0	0 0						
58	Bagdoba	134	0	0 0						
59	Manikgunj	73	0	0 0						
60	Khojpati	30	0	0 0						
61	Khardanga	87	0	0 0						
62	Santola	101	0	0 0						
63	Bhatpukur	25	0	0 0						
64	Goalpara	24	2	8 3	2					
65	Khendwah	91	0	0 0						
66	Khardabazar	42	0	0 0						
67	Sukhahati	33	0	0 0						
68	Goalbari	19	0	0 0						
69	Challa	17	0	0 0						
70	Baynala village	80	0	0 0						
71	Khejurtola	19	0	0 0						
72	Geontala	26	0	0 0						
73	Kumarpukur	45	0	0 0						
74	Gouldanga	59	0	0 0						
75	Kalikapur	105	0	0 0						
76	Ranaputia	23	0	0 0						
77	Kantipota	34	0	0 0						
78	Panchpota	67	0	0 0						
79	Dhela	125	0	0 0						
80	Mirzapur	47	0	0 0						
81	Nutan Deorah	46	0	0 0	..					
82	Sontoshpur	4	0	0 0	.		.			
83	Burkola	25	0	0 0	.					
Total		5,888	17	0 3	5	7	4	1	.	

A NOTE ON TWO INTESTINAL PROTOZOA OF THE INDIAN MONGOOSE

BY

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[Received for publication, March 2, 1931]

From time to time laboratory animals of somewhat unusual type are brought into the Protozoology Department of the Calcutta School of Tropical Medicine and afford interesting material for study. In this way we obtained a small Indian mongoose (*Herpestes auropunctatus*) on the 29th January, 1931. The animal was chloroformed, thin and thick blood films taken from its heart blood, and the intestinal contents searched.

No hæmatozoa were seen in the blood films. [According to Wenyon (1926) a *Babesia* has been described from the Egyptian mongoose, *Herpestes ichneumon* by França, 1908, and one from the larger Indian mongoose *Herpestes edwardsi* by Patton, 1910.]

In the cæcal contents there were found immature oocysts of a coccidium, containing a single rounded unsegmented mass of protoplasm. A moist chamber preparation was put up in a Petri dish, and kept at room temperature, and the development of the oocysts studied. Many of the oocysts went on to the development of two sporocysts, each containing four sporozoites. The oocysts are ovoid in shape and with a double contour wall. They are illustrated in Plate IX.

The average dimensions of the oocysts were 20.6μ in length by 17.2μ in breadth. This parasite does not appear to be a new species, as the dimensions fit in with those of *Isospora rivolta* (Grassi, 1879), a very common parasite of cats and dogs.

Naked eye examination of the small and large intestine failed to show any gross lesion of the mucosa. The Indian mongoose, however, should be added to the already considerable list of hosts known to harbour Isospora infections.

Cultures of the caecal contents were put up in Row's hæmoglobin-saline medium, and also in the liver infusion agar medium recommended by Cleveland and Sanders (1930) for the cultivation of *Entamoeba histolytica*. (We have found that Cleveland and Sanders' medium gives very satisfactory cultures of intestinal flagellate protozoa.) The cultures were incubated at 37°C. After 48 hours there was a rich growth of a typical *Trichomonas*, having four anterior flagella.

This organism is illustrated, as seen in films fixed over osmic-chromic acid and stained by Giemsa's stain, in Plate X. The four anterior flagella beat in unison, whilst the undulating membrane has an entirely different rhythm. In some individuals the axostyle is very prominent, whilst some also show the basal crescentic fibril of the undulating membrane very well. No cysts were seen at any time.

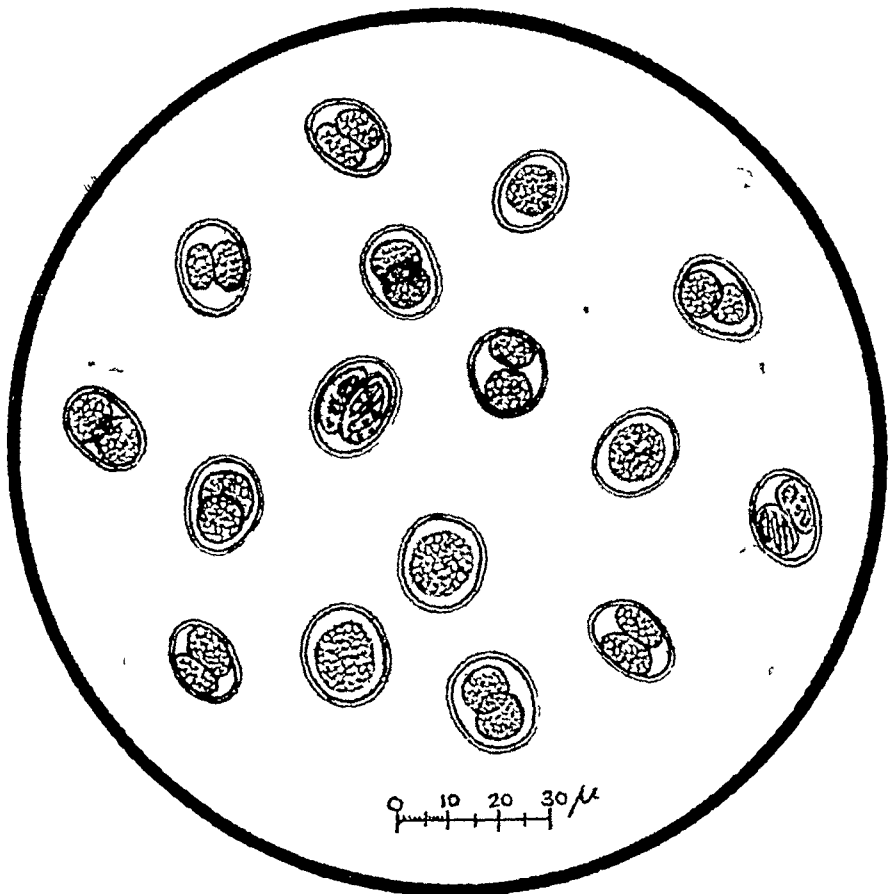
Again, there seems no necessity for creating a new specific name for this parasite, but the Indian mongoose must now be added to the already extremely extensive list of hosts of *Trichomonas* infection.

We are much indebted to Mr. H. Roy, artist at the Calcutta School of Tropical Medicine, for the two accompanying illustrations.

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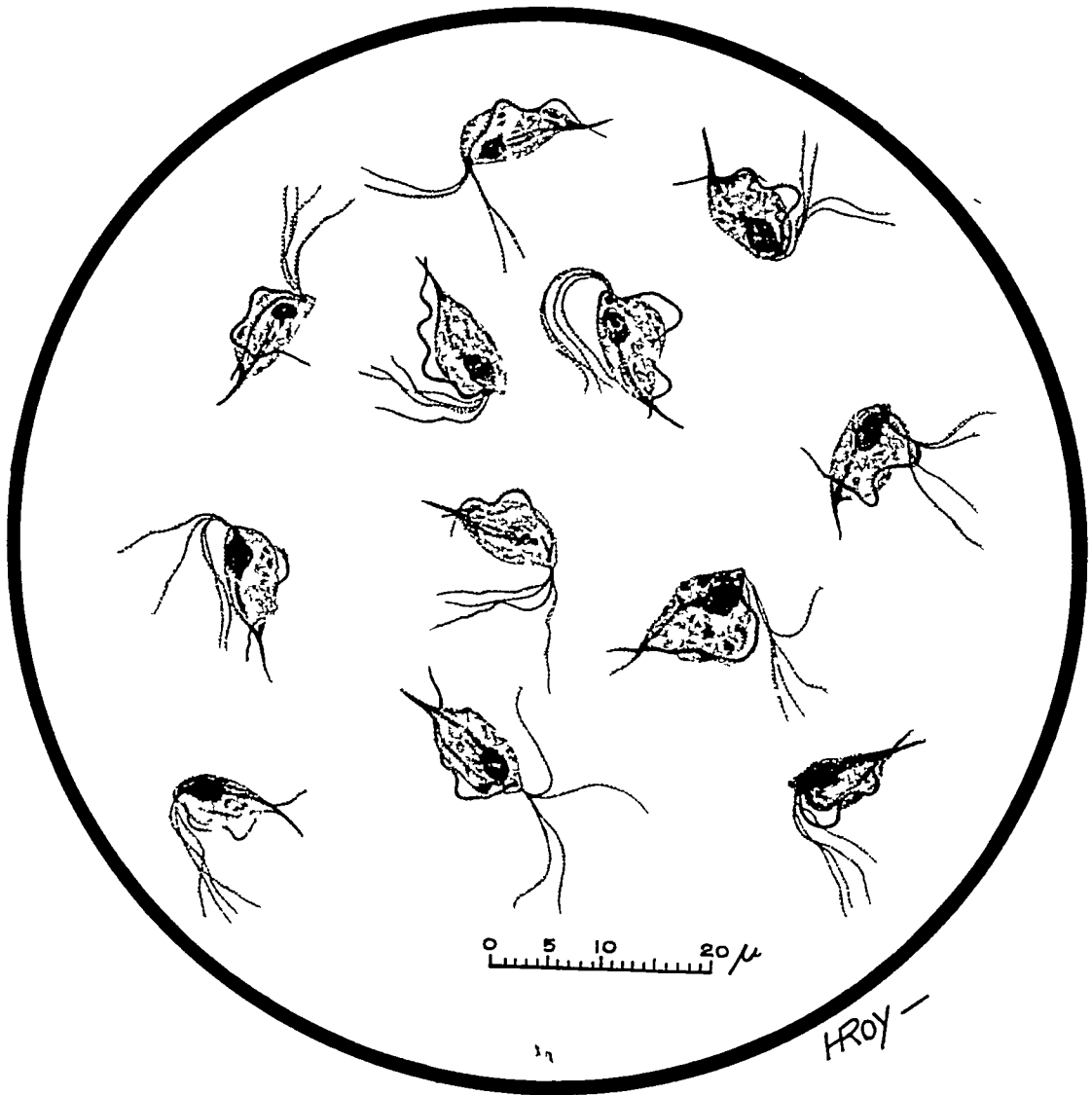
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PLATE IX



Oocysts of an *Isospora* of the Indian mongoose

PLATE X



A Trichomonas of the Indian mongoose

INDIAN EPHEDRAS THEIR CHEMISTRY AND PHARMACOLOGY

BY

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[Received for publication, March 2, 1931]

IN the years following the discovery of ephedrine in 1887, though it received a fair amount of attention from the chemical point of view yet the practical interest in it remained small till the year 1924, when Chen and Schmidt brought out some of its hitherto unknown pharmacological properties arising particularly from its relationship with adienahn. Now that ephedrine has established itself as one of the drugs for the cure of asthma and the allied diseases it became a matter of pharmaceutical and commercial importance to search for suitable sources for its supply, as till recent years, it was considered to be one of the drugs that was difficult to obtain. It was from this point of view that the study of the Indian ephedras was taken up. Recently, however, an extensive trade in the crude drugs has been established between India and the foreign markets, and ephedra is no longer such a rare herb as at one time it was supposed to be.

At least three groups of ephedra species have been studied for their active constituents (a) the Asiatic group consisting of *E vulgaris* and its varieties and *E distachya*, (b) the European group, *E vulgaris* variety *helvetica*, and (c) the American group consisting of six or more species found in southern

California and Mexico None of the American species have been found to contain the alkaloid to any appreciable extent, but the Chinese plant *E vulgaris* contains the alkaloid ephedrine and the European plant yields an alkaloid, isomeric with ephedrine, which has been named pseudo-ephedrine

Up to the year 1928 ephedrine had only been found in any quantity in the Chinese plant, hence the major portion of the drug came from that country. But owing to the disturbed conditions that were prevalent there in 1926-27, interest was taken in its supply from India. In response to an enquiry from the Wellcome Research Laboratories, England, samples of *Ephedra intermedia* were sent by the Conservator of Forests, Kashmir, both to England and to us for analysis, but the results obtained were not very encouraging because of the low yield found in these specimens (about 0.2 per cent). The alkaloid content of the Chinese *Ma-Huang* is supposed to vary from 0.018 per cent to 1.32 per cent, and the two factors that are contributory to these differences are the question of seasonal variation and the influence of altitude (Feng and Read, *Chin Jowr Physiol*, 1928, II, 1, p. 87). Search was, therefore, made for other Indian species of ephedra that may yield higher quantities of the alkaloid. *Ephedra intermedia*, *E Gerardiana* and *E nebrodensis* from various places in northern India (Bashahr Division, Baluchistan, Chakrata, Darjeeling, Hazara, Kashmir, Kangra, Kargil, Kulu, Trans-Frontier territory, Waziristan) have been analysed, and the results obtained indicate that ephedras growing in the drier regions of North-West India contain a high percentage of the alkaloid, and in many cases have shown a higher alkaloidal content than the Chinese species recorded by Read and Feng. In Indian ephedras, as a rule, *E nebrodensis* is the richest in ephedrine content and *E intermedia* the poorest and the highest ephedrine content found is 1.93 per cent. In Indian ephedras the alkaloid content does not increase with the altitude of the locality where the drug grows but is influenced to an appreciable extent by wetness of the locality. These points will be discussed more fully in later parts of this work.

BOTANICAL DESCRIPTION

The genus *Ephedra* belongs to a small but highly developed family (Gentaceæ) of gymnosperms, which are characterized by naked ovules not enclosed in an ovary. There are 30 or more species widely distributed in Central and Western Asia, the Mediterranean Region, the Atlantic Islands, the Southern States of North America, the Andes from Ecuador southwards to Patagonia and the Eastern Argentine. Read and Liu have given an excellent map showing geographical distribution of ephedras all over the world in the *Journal of American Pharmaceutical Association*, XVII, p. 343. They are all rigid, usually much branched, erect or climbing shrubs with scale-like, or rarely filiform or subulate connate leaves in alternating whorls of 2, rarely 3 or 4, sometimes reduced to sheaths. Flowers are small and unisexual and are

aggregated together in few or many-flowered short spikes They may be monœcious or diœcious

The drug is exported in two forms, either tightly compressed into bales, or loosely packed, in this latter form it consists of entire branches which are usually attached to the older stem The main stems are hard and woody, wrinkled longitudinally and covered with cork of a cinnamon brown colour, and have a diameter of 0.05–0.16 inch From the main branches numerous smaller branches of a similar colour arise, and from these the narrow glaucous green stems, which provide the bulk of the drug, are produced Since the green branches arise from about the same height on the main stem, the whole plant commonly has a tufted appearance, but frequently some of the more robust branches again branch at each node The small green branches vary in length from 5–18 inches, the internodes being 0.5–2.5 inches

Brandis (Indian Trees, 1906, p. 686) recognizes 5 species occurring in India, viz, *E. foliata* Boiss, *E. Gerardiana* Wall (Syn *E. vulgaris* Hook, F, in Flora of British India, V, p. 640), *E. nebrodensis* Tineo, *E. intermedia* Schrenk and Meyer, and *E. pachyclada* Boiss Of these *E. nebrodensis* Tineo does not seem to differ from *E. Gerardiana* Wall by any well marked character and is, therefore, sometimes included in the latter Similarly *E. pachyclada* Boiss is considered synonymous with *E. intermedia* Schrenk and Meyer

E. nebrodensis is said to occur in the juniper tracts of Baluchistan (7–10,000 ft), Balti and Lahoul in India In this connection it is interesting to note that the percentage of ephedrine found in *E. Gerardiana*, collected both in Lahoul and Indus territory, is very much higher than the percentage found in samples of *E. Gerardiana* collected from other localities such as Chakrata, Kashmir, Hazara, etc

TABLE I

Showing the difference in the ephedrine contents of *E. Gerardiana* and *E. nebrodensis*

	<i>E. Gerardiana</i>			<i>E. nebrodensis</i>		
	Chakrata, U P (Ephedrine per cent)	Baramula, Kashmir (Ephedrine per cent)	Phari, Tibet (Ephedrine per cent)	Razmak, N Waziristan (Ephedrine per cent)	Shingarh, Baluchistan (Ephedrine per cent)	Lahoul, Kulu (Ephedrine per cent)
May June	0.56	0.39		1.43		1.60
October	0.69	0.54	0.1		1.32	1.93

Furthermore, a sample of *E. Gerardiana* collected from its easternmost limit in Sikkim, where *E. nebrodensis* is not supposed to occur, yielded 0.1 per cent while the Lahoul sample gave as high as 1.93 It is, therefore,

possible that in this case we are dealing with 2 distinct species *E nebrodensis* Tineo having a higher ephedrine content, and *E Gerardiana* Wall with a lower percentage of ephedrine *E nebrodensis* which is sometimes included in the species *E Gerardiana* should, in the opinion of the systematic botanist at the Forest Research Institute, Dehra Dun, be regarded as a distinct species

The following analysis of the species is based on Mr R N Parker's diagnosis in his Forest Flora of the Punjab and Hazara —

Tall scandent shrubs with slender branchlets *E foliata*

Rigid erect shrubs with usually many stems from a stout root stock

Male spikes 1-3 together at the nodes, internodes smooth or slightly rough, rather slender *E Gerardiana*

Male spikes in dense whorls at the nodes, internodes rough, stouter *E intermedia*

E foliata Boiss—Vein Kuchar, Punjab—A tall much branched shrub climbing over bushes and looking like certain species of *Calligonum*. Stem woody, about 3 inches diameter, bark on the branches exfoliating in fibrous shreds, branches slender, usually fascicled, branchlets filiform, dull green in colour, internodes 1-4 inches long. Leaf-sheaths very short with 2 triangular or linear teeth longer than the sheath and often prolonged into narrow linear leaves which may sometimes be up to 1 inch long. Male flowers in sessile or peduncled bracteate spikes which may be solitary or 2-3 together, flowers 6-24 in each spike, bracts rounded, obtuse. Anthers 3-4. Female spikes pedunculate, often in small terminal cymes. Fruit ovoid 0.3 inch long, seeds 2, dark coloured.

Distribution—Baluchistan, Sind, Kurram Valley, Punjab plains, mainly in the southern portion, Salt Range up to 3,000 ft.

E Gerardiana Wall—Syn *E vulgaris* Hook, F, in Fl Br Ind, V, p 640, Vein Tutgantha, Jaunsar—A low, rigid, nearly erect shrub, usually 1-2 ft high, stems up to 1 in diameter, branchlets slender, green, finely grooved, often curved, internodes 0.5 to 1.5 inch long by about 0.05 inch-0.1 inch diameter, striate, smooth or slightly scabrid on the ridges. Leaf-sheaths 0.08 inch long, 2-toothed. Male and female flowers in spikes, usually on separate plants. Male spikes solitary or in pairs, rarely in whorls of 3, flowers 4-8, filaments united in a column protruding from the perianth, anthers 5-8, female spikes solitary, flowers 1-2, each consisting of a single erect, sessile naked ovule. Fruit ovoid, 0.3 inch-0.4 inch long, sweet edible, red when ripe.

Distribution—Himalab District, Kurram Valley, 11,000 ft Himalaya 8-14,000 ft, also in the inner and tracts, ascending in Sikkim to 16,500 ft.

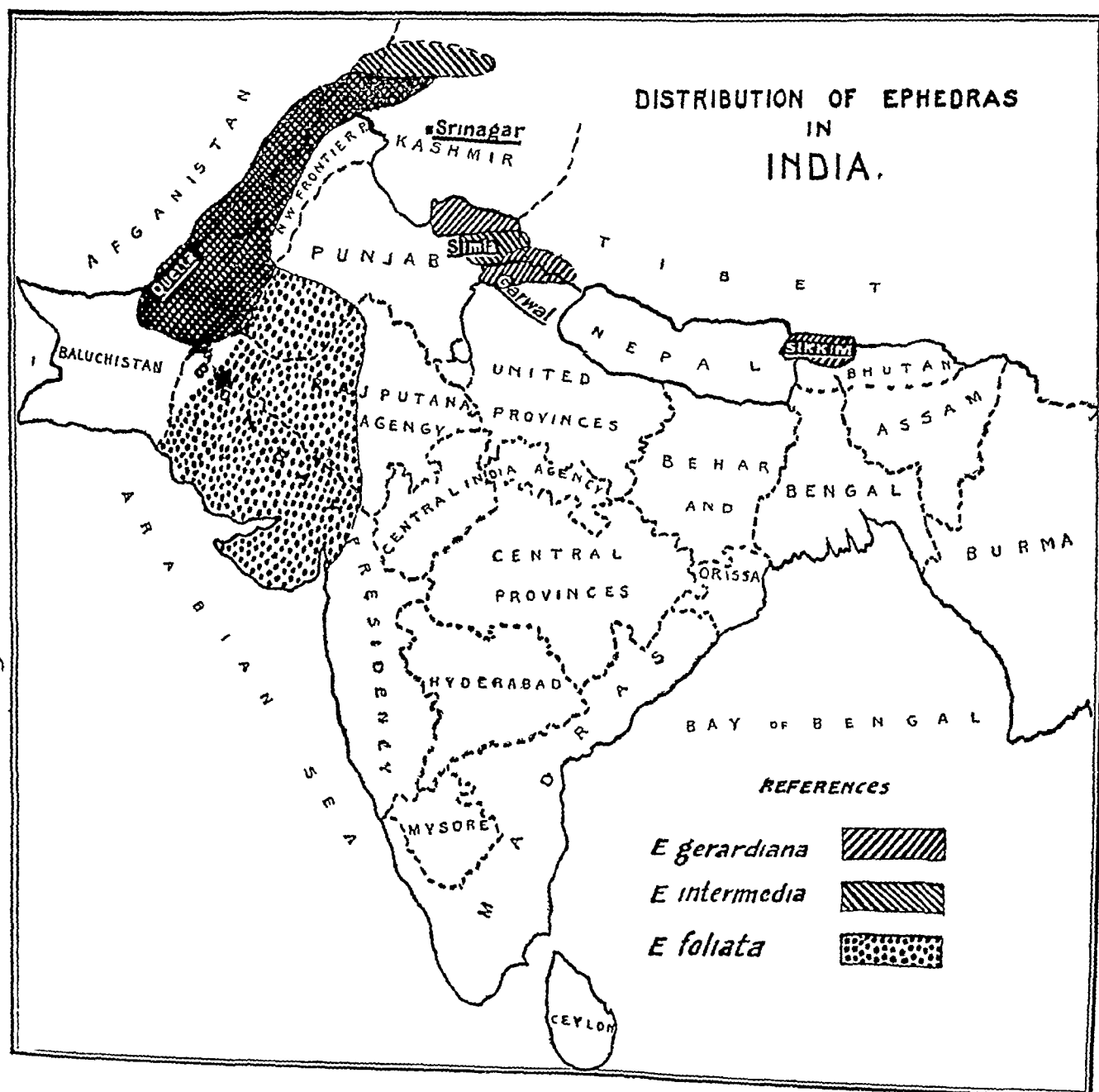
E intermedia Schrenk and Meyer—Syn *E pachyclada* Boiss. Vein Hum, Trans-Indus—A small erect shrub often glaucous, internodes 1-2.5 inch long by 0.08-0.16 inch in diameter, usually more or less rough to the touch when dry. Leaf-sheaths 0.15 inch long or a little less, 2-toothed. Male and

female flowers in spikes usually on separate plant. Male spikes numerous in dense whorls at the nodes, flowers about 8, filaments united in a column protruding from the perianth, anthers 5-6. Female spikes often whorled at the nodes, flowers 2. Fruit 0.3 inch long, ovoid, red when ripe.

Distribution—Baluchistan, N-W Himalaya, chiefly in the inner arid valleys, Chitral 4-5,000 ft on dry rocky slopes, Gilgit, Zaskar Upper Chenab, Kunawar 6-9,000 ft.

Both *E. Gerardiana* and *E. intermedia* are sometimes confused with *Equisetum*—a non-flowering plant, but the latter is never woody, its stems

MAP



are hollow and the leaves which are reduced to the teeth of a sheath are more numerous and at the apex embrace the internodes next to the one from which they arise

DISTRIBUTION OF INDIAN EPHEDRAS

The following table and the Map show the distribution of various species of ephedra growing in India —

TABLE II
Indian Ephedras (Gentaceæ) (Habitat)

Species	Locality	Authority	Remarks
<i>E. foliata</i>	Bombay and Plains of Sind, Salt Range up to 3,000 ft Punjab, Rajputana often gregarious, etc., on the barren desert	Forest flora of Bombay Presidency and Sind by Talbot, Vol II, p 541	
<i>E. peduncularis</i> (<i>E. foliata</i>)	Punjab, Rajputana and Sind	Flora of British India by Hook, F, Vol V, pp 640 and 863	
<i>E. intermedia</i> (Schrenk and Meyer)	Kashmir	Flora of British India by Hook, F, p 863	
<i>E. vulgaris</i> Rich	N W Dry stony hills of Afghanistan, Baluchistan, inner arid and intermediate Himalaya, Jhelum, Chenab and Sutlej 7,800 to 12,800 ft West Tibet to 16,000 ft inner Kumaon and inner Sikkim and adjoining parts of Tibet	Forest Flora of N-W and Central India by Brandis	Syn <i>E. Gerardiana</i>
<i>E. Gerardiana</i>	Kumaon Occurs along the main Himalayan range between 6,500 ft to 14,000 ft Very common on the inner dry ranges bordering Tibet where it grows on open exposed shingly slopes or among rocks	A Flora of Kumaon by Osmaston	Syn <i>E. vulgaris</i>
Do	North Garhwal Divn C Almora, E Almora Very common	Descriptive List of Trees and Shrubs between the Ganges and the Sarda Rivers by Osmaston	
Do	Alpine Himalayas and Western Tibet and Sikkim	Flora of British India by Hook, F, Vol V, pp 640 and 863	
	Temperate and Alpine Himalaya and Western Tibet in the drier regions 7-12,000 ft 12-16,000 ft in Sikkim		

TABLE II—concl'd

Species	Locality	Authority	Remarks
Varieties — Var <i>allichi</i>	Western Tibet, Kima war, Garhwal and Kumaon	Flora of British India by Hooker, Vol V, pp 640 and 863	
Var <i>B savahha</i>	Garhwal and Kumaon	do	
Var <i>R Sikkimensis</i>	Sikkim	do	
<i>E nebrodensis</i> (Tineo) Var <i>procera</i>	L a h o u l and Western Tibet	do	Usually classed with <i>E Gerardiana</i>
<i>E pachyclado</i>	Garhwal From Garhwal Westward ascending to 15,000 ft	do	Syn <i>E intermedia</i>
Var <i>glauca</i>	Mongolia to Kashmir	do	
Var <i>Tibetica</i>	Afghanistan border Western Tibet, Dist Afghanistan	do	
	Behar and Orissa	Botany of Behar and Orissa by Baines	Ephedras not found
	Northern Berar Forests	Descriptive Botanical List, Northern and Berar Forest Circles, C P, by Witt	do
	Central Provinces	Descriptive List of Trees, Shrubs and Economic Herbs of the S C C P by Haines	do
	Chota Nagpore	A Forest Flora of Chota Nagpore by Haines	do
	Gangetic Plains	Flora of the Upper Gangetic Plain, Pts I, II and III, by Duthie	do
	United Provinces, Eastern Circle	Descriptive List of Trees and Shrubs of E C, U P, by P C Kanjwal	do
	Chittagong and Hill Tracts	List of Plants of the Chittagong and Hill Tracts by Heinig	do
	Darjeeling Dist	Trees, Shrubs and Large Climbers found in the Darjeeling Dist by Gamble	do
	Bengal	Bengal Plants by Prain	do
	Upper Assam and Khashi Hills	Preliminary List of Plants of Upper Assam including Khashi Hills by U N Kanjwal	do
	Nilgiri and Pulney Hill tops	The Flora of the Nilgiri and Pulney Hill tops by Pyson	do

EXTRACTION, ESTIMATION AND ASSAY

Although the alkaloid ephedrine was isolated as early as 1887 it was not till recently, when its therapeutic value became known and its demand in the market increased, that greater attention was paid to the methods of its extraction and assay. Feng and Read (*Jour Amer Pharm Assoc*, 1927, XVI, p 1034) have made a critical study of the various methods of assay for ephedias. This study was taken up because of the two peculiarities in ephedrine that had been noticed, firstly that ephedrine is a stronger base than ammonia and secondly that when ephedrine is shaken out with ammonia and chloroform there is a reaction so that the hydrochloride is obtained instead of the free alkaloid. Hence the need for revising the U S P belladonna method became apparent. Feng and Read from their experiments concluded that unless a very large excess of ammonia is employed it will not completely liberate the alkaloids from the acid solution. In our preliminary work we also employed the U S P belladonna method, but were unable to obtain concordant results of titration. Investigation revealed the fact that when the chloroform solution of the drug is evaporated, an appreciable amount of it is simultaneously converted into its hydrochloride. This conversion is not always uniform, hence the discordance in the titration results noted above. This observation was first made by Peterson (*Ind Eng Chem*, 1928, XX, p 388), who found that the action of chloroform on ephedrine is not quantitative, and that ephedrine hydrochloride may or may not be formed when the base is dissolved in chloroform and the solvent is allowed to evaporate. In view of its importance in the assay method, the reaction has now been studied in greater detail. Quantitative experiments show that prolonged contact of chloroform with ephedrine at room temperature (15°–18°) results in its conversion into hydrochloride to the extent of 42 per cent, and that the reaction is accelerated by heat, and retarded by small quantities of alkali carbonates. Other chlorinated compounds, such as carbon tetrachloride, tetrachloroethane, and dichloroethylene, also react much in the same way. This conversion of the base into its hydrochloride takes place not only with the natural ephedrine, but also with the synthetic preparation 'ephedrine'. It is difficult to explain why Feng and Read (*loc cit*) and Chopra and his collaborators (*Ind Jour Med Res*, 1928, XV, p 889) failed to effect this conversion of ephedrine into its hydrochloride on treatment with chloroform. Besides this conversion, a considerable amount of decomposition of the alkaloid takes place, benzaldehyde being one of the products. It is evident, therefore, that chloroform cannot be regarded as a suitable solvent for the extraction of ephedrine. Ether, in fact, is more satisfactory (Moraw, *Jour Amer Pharm Assoc*, 1928, XVII, p 431).

Objection has been raised by Feng and Read (*loc cit*) to the use of ammonia for extraction of the alkaloid on the ground that it does not completely liberate ephedrine from its acid solutions. It has been found during the present work that ammonia is just as effective as sodium and potassium carbonates, provided the aqueous solution after alkalinization is saturated with

common salt to reduce the solubility of the base in water This is shown by the following experiments

ALKALINIZATION WITH AMMONIA

Ephedrine hydrochloride (0.4107 g) was dissolved in 50 c.c. of water, made alkaline with ammonia, and saturated with common salt The mixture was then extracted with four successive portions of ether, 25 c.c. each On removal of the solvent by distillation, the residue was dissolved in excess of 0.1 N-hydrochloric acid and the excess titrated with 0.1 N-sodium hydroxide, with methyl orange as indicator The amount thus found was 0.410 g of ephedrine hydrochloride Similar results were obtained when either alkali carbonate or sodium hydroxide was employed in place of ammonia

The method of extraction that has given the most satisfactory results consists in cold extraction of the finely powdered drug with a mixture of ether and chloroform, rendered strongly alkaline with ammonia This process not only obviates the difficulties involved in the process of extraction with cold alcohol by percolation, but also eliminates, more or less completely, the red colouring matter which is present in the stems of the Indian ephedras Before proceeding with this method it was necessary to settle one point and it was whether chloroform, even when diluted with ether, should at all be employed for the extraction of the drug since it converts ephedrine into its hydrochloride on prolonged contact The following extractions were made in this connection (a) with the usual ether-chloroform mixture (3 parts ether and 1 part chloroform) plus ammonia, and (b) with ether and ammonia only, subsequent operations being the same in both the cases The following results were obtained —

TABLE III

	Total alkaloids	Ephedrine
Ether Chloroform	1.45	0.91
Ether	1.32	0.90

It becomes evident, therefore, that during the course of extraction chloroform when diluted with 3 times its volume of ether does not convert ephedrine into its hydrochloride especially in an alkaline solution and can be employed with perfect safety in assay of the drug

METHOD OF EXTRACTION AND ASSAY

In examining the Indian ephedras for their ephedrine content the following method of assay was finally adopted as the most suitable One hundred grammes of air-dried (containing about 5 per cent of moisture) and finely

powdered green stems of the drug were treated with 400 c c of a mixture of 3 parts of ether and 1 part of chloroform. After keeping for 2 hours, 50 c c of ammonia (3 parts of 0.880 ammonia and 1 part of water) were added and the mixture was thoroughly shaken and kept overnight. The extract was then filtered and the residue treated twice in the above manner. The combined extracts were distilled to remove the bulk of the solvent, and the residue was extracted with portions of 75, 60, 60, and 50 c c of 1.5 per cent hydrochloric acid. The combined acid extract was filtered, made strongly alkaline with potassium carbonate, and almost saturated with common salt. The alkaloids thus liberated were extracted four times with ether. The ether solution was filtered through absorbent cotton, previously soaked in ether. The bulk of the ether was then distilled and the residue allowed to evaporate at room temperature. Excess of 0.1 N-hydrochloric acid was then added to the residue and the excess titrated with 0.1 N-sodium hydroxide, using methyl orange as indicator. From the titration values the total amount of the alkaloid present was calculated on the basis of pure ephedrine. *

The alkaloids were again extracted with ether from the above solutions that had been titrated, after alkalinization. These were then converted into their hydrochlorides by treatment with alcoholic hydrochloric acid. The hydrochlorides were dried over calcium chloride and caustic potash in a vacuum desiccator and, finally, ephedrine hydrochloride was isolated from the mixed hydrochlorides by treatment with dry chloroform, in which the former is practically insoluble. The pure ephedrine hydrochloride obtained in this manner was dried and weighed. The results given in the following table are, therefore, based on the weight of ephedrine hydrochloride actually isolated from the crude drug. The results of analysis of two samples of Chinese ephedra (*Ephedra equistina* and *E. Sinica*) kindly supplied by Prof. B. E. Read, of Peking Union Medical College, were also analysed under identical conditions, and the results obtained are included in the following table, for comparison.

VARIATION OF THE ALKALOID DUE TO SPECIES

The distribution of ephedra in the world is fairly wide and there are many species known of this plant, but the active principle is found only in a few. The American species usually do not contain any ephedrine, the European plant yields an isomeric substance pseudo-ephedrine, the Chinese and the Indian species contain both ephedrine and pseudo-ephedrine. But the amount of any one of the two alkaloids depends upon the species. A detailed study of this has been made in the case of Indian ephedras and it has been found that in general *E. intermedia* is poor and both *E. Gerardiana* and *E. nebrodensis* are comparatively richer in ephedrine content. The following table gives the total alkaloid and the ephedrine percentage of these three species collected from different localities at about the same time of the year. It is unfortunate that

TABLE IV

Species	Locality of collection	Month of collection	Total alkaloids Per cent	Ephedrine Per cent
<i>Ephedra foliata</i>			0 03	Nil
<i>E intermedia</i>	Razmak (N W Frontier Waziris- tan)	Aug 1928	0 17	0 11
	Datakhel (N W Frontier Waziris- tan)	Sept 1928	0 12	0 09
	Shingarh (Baluchistan)	Sept 1929	0 42	0 19
	Zarghat (do)	Sept 1929	0 90	0 48
	Pangi (Bashahr)	July 1929	1 62	0 07
	Spiti (Kangra)	June 1929	1 20	0 05
	Gilgit (Kashmir)	July 1929	0 67	
	Niabat Astor (Kashmir)	July 1929	0 75	0 08
	Kargil (Kashmir)	July 1929	1 17	0 05
	Chini Range (Bashahr Division)	May 1929	2 33	0 38
<i>E Gerardiana</i> and <i>E nebrodensis</i>	Razmak (Waziristan)	May 1929	1 97	1 43
	Shahidum (Baluchistan)	Aug 1929	1 40	0 98
	Sari (do)	Aug 1929	1 31	0 90
	Shingarh (do)	Aug 1929	1 67	1 12
	Zarghat (do)	Sept 1929	1 34	0 96
	Narang (Kagan)	Aug 1929	1 93	1 30
	Dhattamulla (Kashmir)	Aug 1929	1 22	0 68
	Phari (Tibet Frontier)	Nov 1928	0 29	0 10
	Chakrata (Simla Hills)	Nov 1929	0 93	0 72
	Hazara (N W Frontier Province)	May 1928	0 74	0 48
	Baramula (Kashmir)	Nov 1929	1 28	0 80
	Lahoul	Oct 1929	2 79	1 93
	Plas Kohistan (Trans-Frontier)	Sept 1928	1 14	0 84
	Kagan Valley (Hazara)	July 1928	1 83	1 23
	Kagan (Hazara)	Oct 1929	2 15	1 52
<i>F equisetina</i>	China		1 58	0 98
<i>F Sinica</i>	China		1 28	0 63

TABLE V

Locality	Longitude East	Latitude North	Altitude in feet	Species	Month of collection, 1929	Total alkaloids Per cent	Ephedrine Per cent	Proportion of ephedrine to the total alkaloids Per cent
Sputi (Kangra)	77°-78°	31°-32°	8,000-9,000	<i>E. intermedia</i>	June	1.20	0.05	4.1
Gulgit (Kashmir)	74°-75°	35°-36°	4,890	"	July	0.67		
Nabat Astor (Kashmir)	74°-75°	35°-36°	7,836	"	"	0.75	0.08	10.6
Paugi (Bashahr Division)	78°-79°	31°-32°	8,500	"	"	1.62	0.07	4.3
Kargil (Kashmir)	76°-77°	34°-35°	8,733	"	"	1.17	0.05	4.2
Shingai (Baluchistan)	67°-68°	31°-32°	9,000	"	Sept	0.42	0.19	45.2
Zarghat (Baluchistan)	66°-67°	29°-30°	8,000	"	"	0.90	0.48	53.3
Razmak (Waziristan)	71°-72°	34°-35°	8,500	<i>E. nebrodensis</i>	July	1.70	1.05	61.7
Shabidum (Baluchistan)	67°-68°	30°-31°	8,200	"	Aug	1.40	0.98	70.0
Sari (do)	66°-67°	30°-31°	9,000	"	"	1.31	0.90	68.7
Shingarh (do)	67°-68°	31°-32°	9,000	"	"	1.67	1.12	67.0
Zarghat (do)	66°-67°	29°-30°	9,000	"	Sept	1.34	0.96	71.6
Kardung (Lahoul)	76°-77°	32°-33°	10,000	"	July	2.56	1.63	63.6
Narang (Kagan)	73°-74°	34°-35°	8,000	<i>E. Gerardiana</i>	Aug	1.93	1.30	67.3
Dhattamulla (Kashmir)	74°-75°	34°-35°	4,700	"	"	1.22	0.68	55.7
Chakrata	77°-78°	30°-31°	6,885	"	"	0.28	0.14	50.0

figures for all the samples are not available for the months of October and November, when the ephedrine content is highest. Most of the samples, recorded in Table V were obtained from private collectors and for the sake of convenience the months of July and August were chosen. These months, however, do not give the ideal conditions for comparison, as the influence of rainfall on the alkaloid cannot be neglected, especially in localities (viz., Chakrata) where the rainfall in these months is high. This point has been discussed more fully in another part of this paper.

From the figures given above it is clear that the variation of the alkaloid in the three species is very marked. The difference is not so great, as far as the total alkaloid is concerned, but it is well marked in the proportion of ephedrine to the total alkaloids. In general, *Ephedra nebrodensis* and *E. Gerardiana* appear to contain about 60–70 per cent of ephedrine in the total alkaloids and *E. intermedia* about 10 per cent. The only exception to this is the *E. intermedia* obtained from Baluchistan, which contains a comparatively low percentage of the total alkaloids but a high proportion of ephedrine. *Ephedra intermedia* contains, as a rule, a proportionately high percentage of pseudo-ephedrine, and this is particularly the case with *E. intermedia* from Chini Range, Bashahr Division, Punjab. The results for the proportion of ephedrine in total alkaloids as recorded in this paper are slightly different from those obtained by Read and Feng (*Jour Amer Phar Assoc*, 1928, XVII, p 1189) for Indian ephedras, where *E. intermedia* is shown to contain 30–40 per cent of the total alkaloids. This difference may be explained as due to different method of estimating the amount of ephedrine. In this paper, the percentage of ephedrine given is based on the weight of ephedrine hydrochloride actually isolated from the crude plant and not on the probable percentage of the base indicated by the biuret reaction, developed by Read and Feng (*Chin Jour Physiol*, 1927, I, p 397). For purposes of comparison, in the following table the quantities of alkaloids found in the Chinese, American and Indian ephedras are given —

TABLE VI

Country	African (1)	American (3)			Chinese (5)		Indian (7)			
Species	<i>E. alata</i>	<i>E. neriadensis</i>	<i>E. trifurca</i>	<i>E. Californica</i>	<i>E. Simca</i>	<i>E. equisetina</i>	<i>E. foliata</i>	<i>E. intermedia</i>	<i>E. Gerardiana</i>	<i>E. nebrodensis</i>
Total alkaloids Per cent				0.014	1.315	1.754	0.03	2.33	2.15	2.79
Ephedrine Per cent		<i>Nil</i>	<i>Nil</i>	<i>Nil</i>	1.118	1.579	<i>Nil</i>	0.40	1.52	1.93
Pseudo ephedrine Per cent	1.0	<i>Nil</i>	<i>Nil</i>	<i>Nil</i>	0.263	0.264	<i>Nil</i>	1.8		

REFERENCES

- (1) KELLY (1927) *Jour Amer Pharm Assoc*, p 748
 (2) FERRY (1927) *Ibid*, XVI, p 397
 (3) NIELSON (1928) *Ibid*, XVII, p 427
 (4) READ and FENG (1927) Soc Expt Biology and Med
 (5) *Idem* (1928) *Jour Amer Pharm Assoc*, XVII, p 1189
 (6) *Idem* *Ibid*
 (7) KRISHNA and GHOSH (1929) *Jour Soc Chem Ind*, XII, p 67

Assay of ephedras reported by different workers

Species	Total alkaloids per cent of crude drug	Ephedrine per cent of the total	Author
<i>Ma Hwang</i>	0.31-0.40		Nagai
"	0.02-0.09		Chen
"	0.30		Masucci and Suto
"	0.40-0.86		Schoetzon and Needham
"	0.64-1.43		Williams
<i>E. equisetina</i>	1.75	85-90	Feng and Read
<i>E. Sinica</i>	1.32	80-85	"
<i>E. vulgaris</i>	1.02-1.27	50	Chopra and Dikshit
"	1.65-1.70	70-80	Read and Feng
<i>E. pachyclada</i>	1.80	30-36	Chopra and Dikshit
"	1.15	30-40	Read and Feng
<i>E. intermedia</i> var <i>Tibetica</i>	0.25-0.60		Chopra and Dikshit
<i>E. intermedia</i>	2.33	16-20	Krishna and Ghosh
<i>E. Gerardiana</i>	2.56	60-65	"
<i>E. nebrodensis</i>	2.79	65-70	"

Effect of altitude

In the case of Chinese ephedras it has been noticed that the ephedrine contents varies with the altitude of the locality where the ephedras grows. To settle this point, samples collected from various localities were assayed and the results (Table VII) obtained indicate that in the case of Indian ephedras the altitude has no apparent connection with the ephedrine content.

TABLE VII

Species	Locality	Long E	Lat N	Altitude in feet	Average total alkaloid Per cent	Ephedrine Per cent
<i>E. Gerardiana</i> and <i>E. nebrdensis</i>	Dhattamulla (Kashmir)	74-75	34-35	4,790	1.15	0.65
	Hazara	73-74	34-35	6,800	0.70	0.38
	Chakrata	77-78	30-31	6,885	0.63	0.45
	Zarghat (Baluchistan)	66-67	29-30	8,000	1.20	0.80
	Rizmak (Waziristan)	71-72	34-35	8,500	1.46	0.90
	Sari (Baluchistan)	66-67	30-31	9,000	1.30	0.85
	Kagan	73-74	34-35	10,000	1.90	1.20
	Phru (Tibet Frontier)	89-90	27-28	12,000	0.29	0.10
	Plas Kohistan (Trans Frontier)	73-74	35-36	15,000	1.14	0.84
	Lahoul	76-77	32-33	15,500	2.56	1.60
<i>E. intermedia</i>	Gilgit (Kashmir)	74-75	35-36	4,890	0.67	
	Niabat Astor (Kashmir)	74-75	35-36	7,800	0.76	0.08
	Spiti (Kangra)	77-78	31-32	8,000	1.30	0.07
	Zarghat (Baluchistan)	66-67	29-30	8,000	0.80	0.45
	Phangi (Bashahr)	78-79	31-32	8,500	1.62	0.07
	Kargil (Kashmir)	76-77	34-35	8,733	1.17	0.05
	Shingarh (Baluchistan)	67-68	31-32	9,000	0.38	0.17

Effect of rainfall

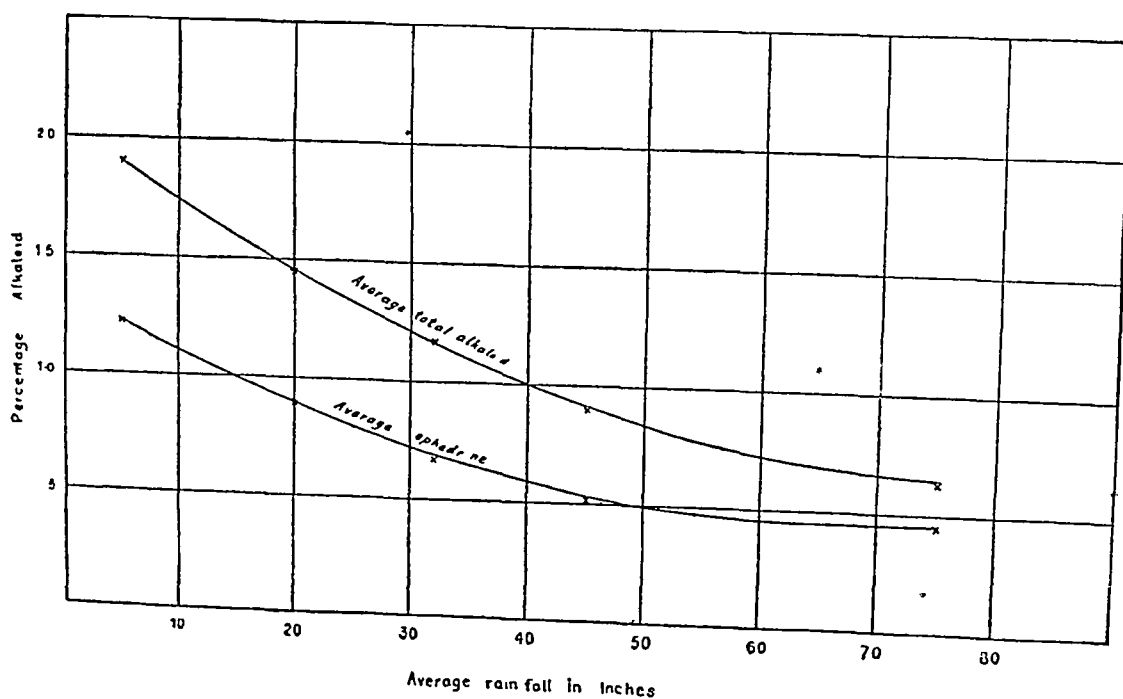
The one factor that has been noticed is that the rainfall of the locality where the ephedras grow seems to have a certain connection with the ephedrine content of the plant. The greater the annual rainfall the smaller is the alkaloidal content. Not only does the annual rainfall affect the average ephedras content, but an occasional heavy shower lowers the ephedrine content considerably. But the effect of an occasional heavy rainfall is only temporary, as the ephedrine content rises again under favourable conditions and reaches its maximum, depending upon the average annual rainfall. This point has been fully discussed under 'Seasonal variation'.

TABLE VIII

The effect of rainfall on the ephedrine content of Indian ephedras

Locality	Average annual rainfall Inches	Average total alkaloids Per cent	Average ephedrine Per cent
Kagan	3-10	1 90	1 20
Razmak	20	1 46	0 90
Kashmir	32	1 15	0 65
Baramula	45	0 90	0 52
Chakrata	75	0 63	0 45

GRAPH 1



SEASONAL VARIATION

It has been shown above that the altitude of the locality where the ephedras grow has no connection with the alkaloidal content of the drug and

that the high rainfall in the locality has a tendency to lower the ephedrine content. In our work on Indian ephedra we have also noticed that the amount of ephedrine found in the plant varies with the time of the year when the collection is made. To study the seasonal variation of the alkaloidal content in ephedras monthly collections of the three species were obtained from different localities in India and assayed. The first collection was made in the month of April, when the plant brings out new shoots, and was carried on through the months when it flowers, till its mature period in October-November, after which it begins to show signs of withering.

Read (*Chin Jour of Physiol*, 1928, II, 1, p 94) from his experiments on Chinese ephedras has concluded 'that there is a progressive increase in the content of ephedrine in *Ephedra Sinica* and *E. equisetina*, so that from spring to autumn there is an increase of about 200 per cent. This strongly supports the old Chinese custom of collecting the drug in the autumn'. From the results recorded in Table IX, it is evident that the variation of the alkaloid from April to November in the Indian ephedras is not so great, nor is the variation so uniform and regular with each month, as shown by Read. In all the cases studied the ephedrine content decreases beginning with the month of May and steadily goes down during the rainy months till it strikes a lowest point in August. From this point onwards the alkaloid increases till it reaches its maximum in October-November and then it falls again during the cold months. The fall in the alkaloidal content during May-August in Indian ephedras cannot be attributed to anything except the climatic conditions. For example, the rainfall of the locality appears to affect the ephedrine content. The heavier the rainfall the lower is the alkaloidal content, so much so, that if a collection is made soon after a heavy rainfall the alkaloidal content is found to be exceptionally low. Such cases have been observed in many places, for instance in Kagan in Hazara where, in September, collection of the drug was made after a continuous heavy rainfall, consequently it showed a very low ephedrine content. Similarly in case of Chakrata the cumulative effect of heavy rainfall in July and August is marked by a lower percentage of ephedrine in the August and September collections. In places like Kagan and Lahoul, where the snow fall takes place early in November, the maximum ephedrine content is attained in October, on the other hand in places like Chakrata, Baranmula, and Chini, the maximum is reached in November. Somewhat similar conclusions have been drawn by Chopra and Dutt (*Ind Jour Med Res*, XVII, 3) from their scanty data on Kashmir ephedras and they record 'that the amount of the total alkaloids gradually increases during the summer months but falls somewhat during the rainy season. It goes up again and attains its maximum in autumn and then it begins to fall again'. This is quite evident from the figures given for the total alkaloid and for ephedrine percentage in Table IX, but it becomes more pronounced when the percentage of ephedrine in the total alkaloid is plotted against the months of collection.

TABLE IX

Seasonal variation of the ephedrine-content of Indian ephedras collected in 1929-30

Month of collection	(1) Chakrata (United Provinces) Long E 77-78 Lat N 30-31				(2) <i>Ephedra Gerardiana</i> Baramula (Kashmir) Long E 74-75 Lat N 34-35			
	Rainfall Inches	Total alkaloids Per cent	Ephedrine Per cent	Per cent of ephedrine in total alkaloid	Rainfall Inches	Total alkaloid Per cent	Ephedrine Per cent	Per cent of ephedrine in total alkaloid
April 1929	1.43	0.81	0.57	70.4				
May "	0.26	0.77	0.56	72.7	3.10	0.66	0.39	59.09
June "					3.12	0.93	0.54	58.06
July "	16.78	0.55	0.36	65.4	2.64	1.13	0.65	57.5
August "	15.78	0.28	0.14	50.0	3.30	1.22	0.68	55.7
September "	2.86	0.55	0.31	56.3	2.40	0.99	0.62	62.6
October "	2.72	0.97	0.69	71.1	1.73	0.75	0.54	72.0
November "		0.93	0.72	77.4	0.92	1.28	0.80	62.5
December "	Snow	0.77	0.56	71.6	2.47	0.83	0.50	60.2
January 1930	"	0.72	0.51	70.8				

(1) Samples were collected during the first week of the month

(2) Samples were collected on the 2nd day of the month

TABLE IX—*contd*

Month of collection	(3)				(4)			
	Kagan (North-West Frontier) Long E 73-74 Lat N 34-35				Lahoul (Kulu) Long E 76 5-77 Lat N 32 5-32 8			
	Rainfall Inches	Total alkaloids Per cent	Ephedrine Per cent	Per cent of ephedrine in total alkaloid	Rainfall inches	Total alkaloids Per cent	Ephedrine Per cent	Per cent of ephedrine in total alkaloid
April 1929	<i>Nil</i>	1 92	1 39	72 3				
May "	1 wet day	1 50	1 00	66 6				
June "						2 56	1 63	63 7
July "	12 wet days	1 50	0 94	62 6				
August "	Continuous rain 23rd- 28th	1 93	1 30	67 3				
September "		1 71	0 99	57 8				
October "	11 wet days	2 15	1 52	70 7	Snow	2 79	1 93	69 1
November "		1 45	0 91	62 7	"	2 36	1 66	70 3
December "					"			
January 1930					"			

(3) Samples were collected on the 1st day of the month Kagan has no meteorological station, rainfall data is therefore approximate

(4) Practically no rainfall from June to October From October it snows

TABLE IX—concl'd

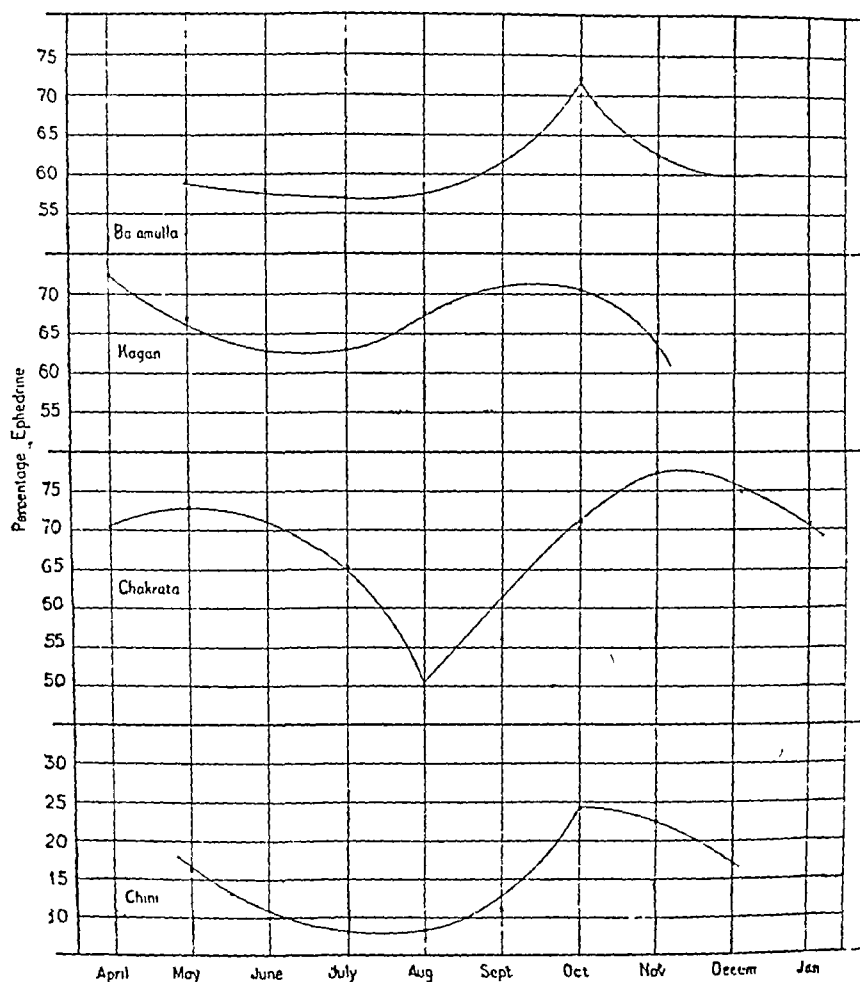
Month of collection	<i>E. nebrodensis</i> (5) Razmak (N Waziristan) Long E 71-72 Lat N 34-35					<i>E. intermedia</i> (6) Chini (Bashahr Division, Punjab) Long E 71-72 Lat N 31-32				
	Rainfall Inches	Total alkaloids Per cent	Ephedrine Per cent	Per cent of ephedrine in total alkaloid	• Rainfall Inches	Total alkaloids Per cent	Ephedrine Per cent	Per cent of ephedrine in total alkaloid		
April 1929					2.40	2.33	0.38	16.3		
May "	1.19	1.97	1.43	72.5	1.86	1.78	0.18	10.1		
June "		1.77	1.13	63.8	1.21	1.62	0.13	8.0		
July "	6.59	1.70	1.05	60.1	2.06	1.78	0.15	8.4		
August "	7.58	0.85	0.51	60.0	2.46	1.76	0.20	11.3		
September ,					1.83	1.12	0.27	24.1		
October "					0.58	1.52	0.34	22.3		
November "					Snow	1.54	0.26	16.8		
December "	Snow	1.00	0.62	62.0	"	1.16	0.07	6.0		
January 1930	"				"					

(5) Samples were collected during 4th week of the month

(6) Samples collected on 1st day of the month, average rainfall for Kilba (Chini Range) which is the nearest meteorological station to Chini is given

GRAPH 2

—Seasonal variation of the percentage of Ephedrine in total alkaloid content of Indian ephedras ,



EFFECT OF STORAGE

A point of industrial interest that has also been studied is the effect of storage on the ephedrine content of the drug. From the results of the analyses given in Table X it appears that if the drug is thoroughly air dried and stored in a dry place to prevent bacterial growth, it can be kept for a sufficiently long period without any diminution in its ephedrine content.

LARGE SCALE EXTRACTION

The limited use of ephedrine is to some extent due to its high price (Rs 55 an ounce in 1928-29, about Rs 30 in 1930) and if by any means prices could be brought down, it will probably find a wider use in medicine. The difficulty that has hitherto attended its extraction has been the fact that the alkaloid is decomposed by heat and is easily transformed in contact with acids.

TABLE X

The effect of storage on the ephedrine content of ephedras

Description	Date of collection	Dates of analyses	Total alkaloid Per cent	Ephedrine Per cent
<i>E. intermedia</i> from China	Nov 1928	March 1929	2 08	0 50
		Dec 1929	1 99	0 48
<i>E. Gerardiana</i> from Kashmir	June 1928	Aug 1928	0 86	0 55
		June 1929	0 76	0 47
		Dec 1929	0 83	0 50
Do	Oct 1928	Nov 1928	0 93	0 63
		June 1929	1 01	0 67
		Dec 1929	0 92	0 60

into the isomeric alkaloid pseudo-ephedrine. In order to prevent its formation it is usual, therefore, to extract the drug by chloroform, alcohol or ether in the cold. Complete recovery of these solvents, after extraction, is never possible, hence the loss in the solvent raises the price of the drug. For large scale extraction any method necessitating the use of expensive solvents cannot be advocated. An attempt was, therefore, made to extract the alkaloid by means of dilute hydrochloric acid in cold solution. Feng and Read (*Jour Amer Pharm Assoc*, 1927, XVI, 1035) employing hydrochloric acid for extraction succeeded in obtaining only about 0.3 per cent of the alkaloids from a sample containing 1.32 per cent of total alkaloids. Using dilute acid (0.5 per cent) it is now found possible to extract the alkaloid completely. No decomposition of the alkaloid was observed to take place when the acid extract was neutralized with sodium carbonate and later evaporated on a water bath to a small bulk. The following table gives the results obtained in this manner —

TABLE XI

	Total alkaloid Per cent	Ephedrine Per cent
Extraction with 0.5 per cent HCl	1.33	0.60
Extraction with ether and chloroform	1.28	0.63

These results indicate that the conversion of ephedrine into pseudo-ephedrine does not take place in contact with hydrochloric acid of the above concentration and at a temperature of 25°C, as has hitherto been supposed.

The following method gave the most satisfactory results of extraction of ephedrine on a large scale. One kg of the finely-powdered plant (consisting of green twigs only) was macerated with 5 litres of cold 0.5 per cent hydrochloric acid and kept overnight, and the acid extract was then squeezed out. The operation was repeated twice, using 3 litres of acid each time. The mixed acid extract (11 litres) was filtered and neutralized with sodium carbonate till it was just neutral to Congo, but acidic to litmus. It was then evaporated on the water bath to about 800 cc, and later made strongly alkaline with sodium carbonate. The precipitate formed was separated and washed with a little water and the washings were added to the filtrate. To the filtrate, which contained the bulk of the alkaloids, a large amount of common salt was added, and the whole was extracted four times with ether. The solvent was removed by distillation. The alkaloidal residue was then converted into the hydrochloride by neutralizing it with dilute hydrochloric acid. The solution was concentrated by evaporation at 45°C–50°C when the hydrochlorides crystallized out and these were treated with dry chloroform to dissolve the hydrochlorides of other bases present (chiefly pseudo-ephedrine). Pure ephedrine hydrochloride thus obtained was dried and weighed. A further quantity of ephedrine was recovered from the precipitate, previously removed, by boiling with benzene.

Chemistry of ephedrine and pseudo-ephedrine

Ephedrine $C_{10}H_{15}ON$, is colourless, crystalline substance mpt 41°C–42°C. The hydrochloride forms colourless needles mpt 216°C. $(\alpha)_D^{25} = -34.2^\circ$ in water and $(\alpha)_D^{25} = -6.81^\circ$ in absolute alcohol. The platinumchloride $(BHCl)_2PtCl_4$ crystallizes in colourless needles mpt 186°C.

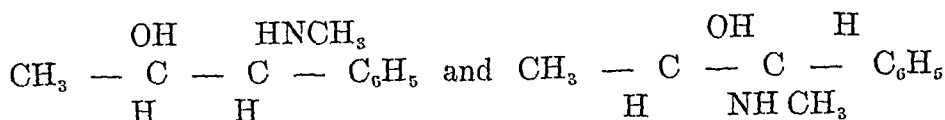
Pseudo-ephedrine or iso-ephedrine $C_{10}H_{15}ON$ occurs with ephedrine in *Ephedra Gerardiana* and *E. intermedia* and is formed by heating ephedrine with hydrochloric acid. $(\alpha)_D^{25} = 50$. It is a dextro-rotatory isomer of ephedrine and crystallizes from ether and melts at 114–115°C. The base is a white colourless crystalline substance occurring in form of long needles, freely soluble in alcohol. The hydrochloride forms colourless needles mpt 176°C. Forms a remarkable soluble oxalate in contrast to the sparingly soluble ephedrine oxalate.

The oxalate of ephedrine crystallizes from water in fine needles sparingly soluble in H_2O and less so in alcohol. This relative insolubility of ephedrine oxalate provides a fairly simple means of separating alkaloid from the associate to isomer d-pseudo-ephedrine.

The ratio of ephedrine to d-pseudo-ephedrine seems to vary with the different species, the real value of the herb being determined by a high γ -ephedrine content.

The alkaloid ephedrine can exist in no less than six forms γ -ephedrine, d-ephedrine, d γ -ephedrine, γ -pseudo-ephedrine, d-pseudo-ephedrine and d γ -pseudo-ephedrine.

Emde (*Arch Pharm*, 1907, 245, 662) has suggested that ephedrine and pseudo-ephedrine are optical isomerides, the relationship between the two being the same as that between L ascorbic acid and L ribonic acid



After the separation of the alkaloids γ ephedrine and d-pseudo-ephedrine there remains a small precipitate of oily residue which is still high in alkaloid content. From this oily residue Sydney Smith has separated two additional alkaloids γ -methyl ephedrine and nor-d-pseudo-ephedrine. γ -methyl ephedrine was prepared by distilling the oily residual alkaloids under reduced pressure and purified through the alcohol soluble oxalate, γ -methyl ephedrine gives an optical rotation $(\alpha)_D - 29.2$

The alkaloids γ -ephedrine and d-pseudo-ephedrine are not particularly sensitive to potassic mercuric iodide solution. On the addition of that reagent to a 1 per cent neutral solution of the sulphates of the alkaloid no precipitate occurs. Both alkaloids are precipitated in a 3 per cent neutral solution but the precipitate is readily soluble in dilute acids. To the same reagent γ -methyl ephedrine and γ -d-pseudo-ephedrine behave in a marked contrast to the above. They are readily precipitated from a 1 per cent of neutral solution of the sulphates, the precipitate remaining undissolved on the addition of dilute acid (*Smith Pharm Jour*, 1929, p 606)

Probably the most important property of ephedrine is its stability, its solutions are not decomposed by light, air or heat, and age apparently does not affect their activity. Thus a solution of ephedrine hydrochloride, prepared and sealed in a sterile ampoule for 6 years showed no change in appearance and produced the customary pressor response when injected into a pithed cat ('Chen and Schmidt-Ephedrine and Related Substances,' 1930, p 13). Kendall and Witzmann (1907) have demonstrated the great resistance of ephedrine to oxidation as compared with epinephrine: the former is not oxidized by dibromophenolindophenol, methylene blue, or indigo carmine, whilst the latter is oxidized by all these reagents. Pseudo-ephedrine hydrochloride is also very stable, a 1 per cent solution still retains its properties after keeping at room temperature for many weeks and it is believed may keep indefinitely without deterioration. Its solutions can be boiled without decomposition. Mixing with sera does not interfere with the activity of either ephedrine or pseudo-ephedrine even after incubation for many hours.

The most serviceable qualitative reaction of ephedrine is that with copper sulphate and sodium hydroxide which was first pointed out by Nagai (1892). A purple colour appears which is extractible with ether. This test is sensitive to one part of ephedrine in 400, and if the concentration exceeds 1 in 40 a pinkish purple precipitate is formed and this is completely soluble in ether.

Action of chloroform and related compounds on ephedrine and pseudo-ephedrine

In an earlier part of this paper it has been pointed out that when natural ephedrine or the synthetic preparation 'ephedrine' is allowed to remain in contact with chloroform for some time it gets converted into its hydrochloride with slight decomposition. This reaction has now been studied in greater detail and it has been found that the reaction is accelerated by heat and retarded by small quantities of alkali carbonates and that other chlorinated compounds such as carbon tetrachloride, tetrachlorethane, dichloroethylene, also react much in the same way. The following experiments were performed to show the amount of conversion of the base into its hydrochloride and the amount that is decomposed —

In the following experiments pure ephedrine hydrochloride, m.p. 216°C (α)_D — 34.2° (in water), and ephedrine, m.p. 41° – 42°C (α)_D — 6.81° (in absolute alcohol), were employed. A small quantity (0.3–0.5 g) of pure crystallized ephedrine was dissolved in about 50 c.c. of the pure solvent, and kept at 40° – 45°C till the solvent had evaporated. During this process a large quantity of needle-shaped crystals had separated. These were found to consist of ephedrine hydrochloride (m.p. 212° – 214°) and were removed, washed with ether, and weighed. The titration of the ether washings after removal of the solvent gave the amount of the free base, and the balance represented the amount that had decomposed into benzaldehyde and other products. The following table gives the results obtained —

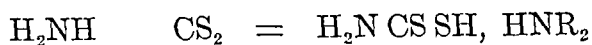
TABLE XII

No	Experiment	Conditions of experiment	Base recovered Per cent	Change into hydrochloride Per cent	Amount decomposed Per cent
1	Base—chloroform	Evaporated at 40°C	25.0	42.0	33.0
2	Base—chloroform 0.1 per cent ammonia	Spontaneous evaporation at room temperature	22.5	43.7	33.8
3	Base—chloroform	Heated in sealed tube at 120° for 5 hours	16.5	55.3	28.2
4	Base—chloroform 0.01 g sodium carbonate	" "	46.8	26.5	26.7
5	Base—pure sulphur free carbon tetrachloride	Evaporated at 40°C		64.0	
6	Base—tetrachloroethane	" "	4.6	81.0	14.4
7	Base—dichloroethylene	" "	61.7	10.4	27.9
8	Ephedrine—chloroform	" "	47.2	20.4	32.4
9	Ephedrine—carbon tetrachloride	Evaporated at 40°C	5.4	64.3	30.3
10	Ephedrine—tetrachloroethane	" "	4.6	70.1	25.3
11	Ephedrine—dichloroethylene	" "	56.8	12.7	30.5

If, however, the base after extraction with chloroform is titrated immediately, the amount obtained is more or less quantitative. For example, 0.4730 g of ephedrine hydrochloride was dissolved in water, rendered strongly alkaline with potassium carbonate, and the liberated base was extracted with four successive portions (75, 60, 60, 60 c.c.) of chloroform. The chloroform extract was filtered through absorbent cotton, previously soaked in chloroform, into a stoppered cylinder, 50 c.c. of water were added to the mixture, and was immediately titrated with 0.1 N-hydrochloric acid. The amount of the acid neutralized was found to be equivalent to 0.3739 g of ephedrine, i.e., 26.4 per cent of the ephedrine hydrochloride employed.

The action of carbon disulphide on natural ephedrine and ephetonin

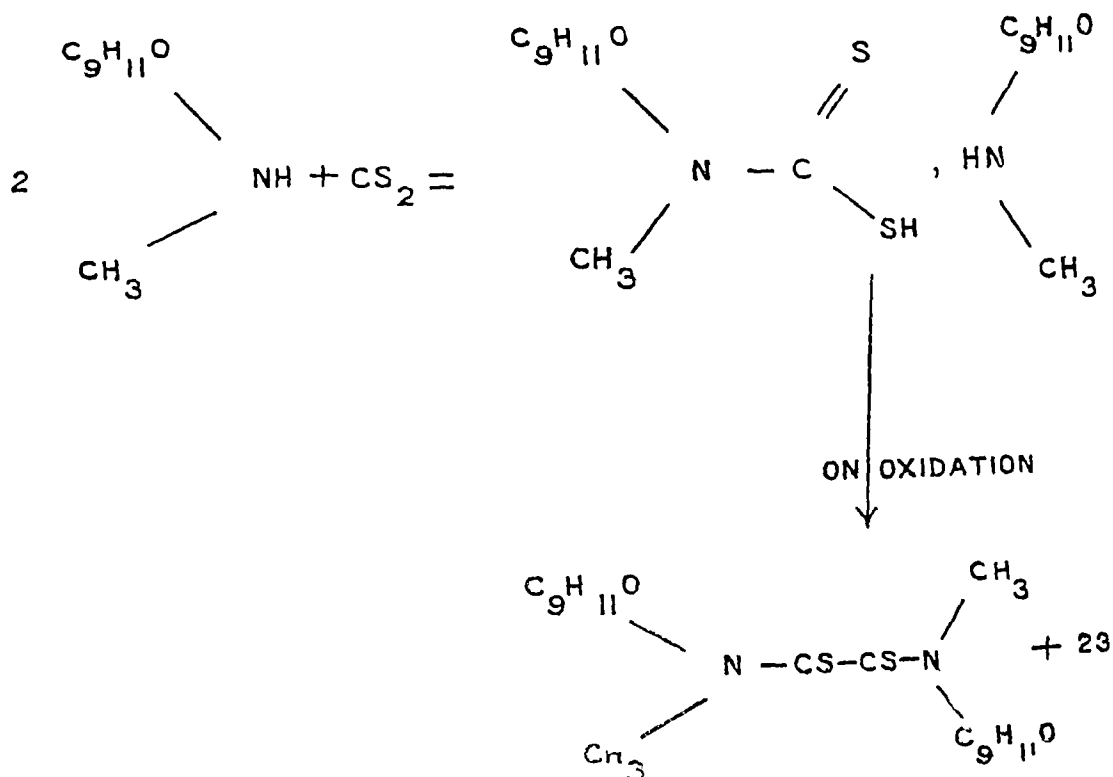
Ephedrine and ephetonine like the other simpler secondary amines react with carbon bisulphide and other related compounds, forming salts of alkyl dithio-carbamic acids such as the following —



These salts are very readily formed in the cold, by mixing one molecular proportion of carbon disulphide in ether with two molecular proportions of ephedrine, also in the same solvent, when in about five minutes a white precipitate makes its appearance and rapidly sets to a gelatinous mass. During the reaction there is rise of temperature and if a fair excess of the solvent is not employed, there is always a danger of the reaction remaining incomplete, otherwise the product is obtained in correct theoretical yields. In fact, the reaction is so quick and reliable that a method for the detection and estimation of small quantities of ephedrine can be evolved. The gelatinous mass on filtration can be crystallized from water or a mixture of absolute alcohol and petroleum ether in long, shining needles, m.p. 140°C. With ephetonine, under identical conditions, a compound of melting point 110°C is formed. Analytical results are as follows: Found S = 15.96 per cent, N = 6.72 per cent, formula $\text{C}_{21}\text{H}_{30}\text{O}_2\text{N}_2\text{S}_2$ requires S = 15.76 per cent, N = 6.89 per cent.

If treated with dilute acids, the above compound at once breaks up into the corresponding salt of ephedrine and globules of carbon disulphide make their appearance on the surface. This is not only the case with solutions of strong acids but even with such acids as oxalic or perchloric, the behaviour is similar. If to a chloroformic solution of the compound any bromine is added, at once a thick crystalline precipitate is formed and this has been found to be the hydrobromide of ephedrine. On heating to 150°C, it loses sulphuretted hydrogen getting converted into a thick oil. And if an alcoholic solution is treated with powdered potassium ferricyanide, white shining plates make their appearance, on standing for about 48 hours. These can be easily separated, purified from the accompanying sulphur, and on recrystallization from dilute alcohol melt at 120°C. Analytical results are S = 15.68, formula

$C_{22}H_{28}O_2N_2S_2$ requires S = 15.38 per cent. The above reactions are shown graphically by the following —



Compounds like the above are also formed when ephedrine, pseudo-ephedrine and ephetonine are reacted with potassium xanthate, hydrogen sulphide and mercaptans. These are under further investigation and will form a part of a separate communication.

EXPORT OF EPHEDRAS

Work carried out by us has undoubtedly established the commercial values of *E. Gerardiana* and *E. nebiodensis* and has shown that the Indian species are quite as rich in ephedrine content, if not in some cases richer, as the Chinese species. Already a demand for Indian ephedras has been created in India and elsewhere. It is difficult to get exact statistics of the exportation of any particular drug material because drugs are generally classed together in the customs returns. At a conservative estimate it may be said that about 2,000 maunds of ephedra were exported from India during 1928-29. These figures represent only a fraction of the trade which has recently been developed in China. The figure for export from the whole of China is about 8,000 maunds a year.

EPHEDRINE IN OTHER INDIAN PLANTS

Chopra and De (1930) showed the presence of a sympathomimetic alkaloid in *Sida Cordifolia* whose pharmacological action closely resembled that of ephedrine and they thought that the alkaloid was undoubtedly ephedrine. Later Ghosh and Dutt (1930) showed that the sympathomimetic alkaloid referred to above showed all the chemical and physical characteristics of ephedrine. This plant is distributed throughout the tropical, sub-tropical India and Ceylon growing wild along the roadside. The roots, leaves and seeds are all used in the Hindu medicine as a stomachic and cardiac tonic. The whole plant (including leaves, seeds, stems and roots) contains the alkaloid to the extent of 0.085 per cent. The seeds contain much larger quantities of 0.32 per cent. The interesting point about this work is the occurrence of ephedrine in two entirely different groups of the vegetable kingdom as the ephedras belong to the group of *gymnosperms* while *Sida Cordifolia* belongs to *angiosperms*.

Chopra and De (unpublished) have also found the presence of a sympathomimetic alkaloid resembling ephedrine in *Moringa Pterygosperma* (Sajina) and it will be interesting to see when their results are published if it is really ephedrine or some other alkaloid.

PHARMACOLOGY OF INDIAN EPHEDRAS

Few drugs of recent years have attracted so much attention of the medical profession as the alkaloid ephedrine. A large amount of experimental work has been done and the well compiled bibliography by Professor Read shows the interest which it has evoked. Although the plant has a world-wide distribution it is interesting that it was not used in medicine except in China. In that country there is evidence to show that the drug was in use five thousand years ago. It is curious to note that although a number of active species of ephedra grow in India, the plant appears not to have been used in the indigenous medicine in this country. Aitchinson describes that some parts of *E. vulgaris* were used medicinally in Lahoul, but inquiries show that the plant has not been mentioned in the Hindu (Ayuvedic) or Mohammedan (Tibbi) systems of medicine. It has been alleged that a variety of ephedra, probably *E. intermedia* is the famous *Soma* plant from which the ancient Hindu Rishis (ascetics) prepared their favourite drink, but there is little evidence to support this fact.

The powerful sympathomimetic effects produced by the alkaloid ephedrine and its gradually increasing use by the medical profession induced one of us (R N C) to explore the resources of the Indian varieties in 1926 and to investigate their chemical composition, pharmacological action and clinical uses. Later two of us (S K and T P G) made a very thorough chemical study of different varieties under varying conditions under the auspices of the Forest Research Institute, Dehra Dun, and results of this work have been stated above.

While studying the chemical composition and pharmacological action of the Indian varieties, it was discovered that the pseudo-ephedrine content of many of the Indian varieties was remarkably high. We, therefore, not only studied the action of ephedrine isolated from the Indian varieties, particularly with regard to the aspects to which little attention had been paid by other workers, but we made a very detailed study of the alkaloid pseudo-ephedrine whose action was said to resemble that of ephedrine. This was considered specially important as the retail price of ephedrine was about Rs 1,100 per pound then and even at that sufficient quantities required were not available. The alkaloids for this investigation were isolated from such Indian varieties as *E. vulgaris*, *E. intermedia* and *E. nebiodensis* by the Department of Chemistry of School of Tropical Medicine and were specially purified.

Action of ephedrine from Indian ephedra

The action of the alkaloid ephedrine has been investigated by many workers and we found that the ephedrine isolated from the Indian ephedrine to have similar properties and action. We, therefore, paid attention to the points which had not been fully gone into by other workers.

It is generally agreed that the pressor effects produced by ephedrine are due to the vaso-constriction produced by stimulation of the sympathetic nerve-endings and ganglion cells. Although the stimulant action on the sympathetics has been studied in detail, very little attention has been paid to the action of the alkaloid on vagal mechanism. Chen and Schmidt (1925) observed that the threshold electrical stimulation of the vagus caused cardiac inhibition during the acceleration produced by ephedrine. Chen and Meek (1928) found that ephedrine usually slowed the pulse rate in stimulating doses, when given intravenously, intramuscularly or orally in anaesthetized and non-anaesthetized animals with intact vagi. Acceleration always occurs, when the vagi are paralysed with atropine. In larger doses the rate is slowed irrespective of the vagal control.

During our experiments with this alkaloid which were chiefly conducted on cats, we found that although the drug produced the usual marked and persistent rise of blood-pressure, the effect on the auricles and the ventricles is contrary to the findings of many investigators. In our experiments the auricles showed an increase in amplitude of contractions, the ventricles showed a very slight decrease in the amplitude of individual contractions instead of the stimulant effects observed by many writers. There was no change in the frequency of the beats of either chambers of the heart. When larger doses of the alkaloid were administered the amplitude of contraction of both the auricles and the ventricles decreased. The prolonged rise of blood-pressure, without any stimulation of the ventricle, suggests action on the vaso-motor system. If sufficiently large doses of nicotine are given to paralyse the sympathetic ganglion cells, ephedrine still produces rise of blood-pressure but much less as compared with the effect produced when these cells are intact.

The paralysis of the sympathetic ganglia appears to have decreased the pressor response of the drug. If vaso-motor sympathetic nerve-endings are paralysed with ergotoxine, the pressor response is absent and the effect on the auricles and the ventricles is one of very slight depression. The cause of rise of the blood-pressure would, therefore, appear to be (a) stimulation of sympathetic nerve terminals and (b) sympathetic ganglion cells but chiefly the former.

Why is it that the stimulating action of the alkaloid on the heart is not seen when the drug acts by stimulating the sympathetic? Ephedrine not only stimulates the vaso-motor fibres but also the accelerator fibres in the heart, as is shown by the fact that if an isolated heart is perfused by 1 in 100,000 dilutions of the alkaloid, there is a marked augmentation of the force and frequency of the heart. The accelerator effect in intact animals, therefore, must be counterbalanced by some other factor or factors. Ephedrine does not act on the vagus centre in the medulla because when an animal is pithed and its brain including the cerebrum, cerebellum and the medulla are destroyed, the results produced do not materially differ than when the centres were intact. Section of the vagi also does not alter the response of the auricles, ventricles or the blood-pressure produced by ephedrine. The effect, therefore, cannot be central but is peripheral. The alkaloid produces a distinct increase in the amplitude of contractions and frequency of beat of both the auricles and the ventricles after paralytic doses of nicotine to sympathetic ganglia. The same phenomenon is observed after the vagus terminals have been paralysed by atropine. It would, therefore, appear that the peripheral inhibitory mechanism, i.e., the terminations of the vagi as well as the ganglia are stimulated by ephedrine.

Besides this the irritability of the heart muscle is distinctly depressed by the alkaloid especially when large doses are given. The latent period is not affected in a frog's heart and the refractory period not altered in the mammalian heart.

The action of ephedrine, therefore, is the sum total of these factors, viz., (1) stimulation of the vagus, (2) stimulation of the sympathetic and (3) in larger doses depression of both the auricles and the ventricles by its direct action on the heart muscle.

The effects produced by ephedrine in myocardiograph experiments above described resemble those produced in an animal by compressing the aorta, the contraction of the arterioles due to vaso-motor stimulation produced by the alkaloid produces similar effects to artificial compression. The apparent stimulation of the auricle produced in these experiments is in all probability produced by distension of the auricle brought about mechanically owing to the right auriculo-ventricular valve not closing properly and allowing some of blood to regurgitate into the auricle during the ventricular systole. The auricle thus becomes distended and the stretched auricular muscle responds to the increased auricular pressure by contracting more forcibly. There is thus an increase in amplitude of the contractions while there is no change in the

frequency of the heart beat. If the stimulation was produced through the agency of the accelerator mechanism, the force and frequency of the heart as a whole including the ventricle would have been accelerated.

The direct depressant action of the alkaloid on the myocardium is better seen when the injections are repeated. Chen and Schmidt have shown that the first injection of ephedrine produces a well marked pressor effect, with the second injection this rise of blood-pressure is very slight, while subsequent injections produce very slight effect. That is the reason why auncles do not show any stimulation after the second and subsequent injections.

Action of pseudo-ephedrine

Although pseudo-ephedrine was discovered by Meick in 1893, it has not received the same attention from investigators as ephedrine. A comparative study of the pressor action of ephedrine and pseudo-ephedrine has been undertaken recently by various workers who are all agreed that the former alkaloid is more powerful than the latter in raising the blood-pressure. Read and Lau (1926) found pseudo-ephedrine is half as strong as ephedrine in this respect. A perusal of the literature shows that while some work has been done on the pressor properties of pseudo-ephedrine, the detailed action of this alkaloid has not been investigated. This was, therefore, taken up.

Local effects and absorption—The alkaloid does not set up any marked local reaction when injected subcutaneously or intramuscularly or instilled into the conjunctival sacs. It is readily absorbed from the site of injection as well as from the gastro-intestinal tract. After absorption from the intestine pseudo-ephedrine produces the same physiological action as when it is introduced direct into the circulation by intravenous injection. This is shown by the fact that injection of the alkaloid into one of the mesenteric veins produces the same degree of rise of blood-pressure as when the injection is made into one of the systemic veins. The liver does not appear to have any detoxicating action on this alkaloid as is the case with ephedrine. This is the reason why both these alkaloids are as effective, when given by the mouth in purified condition or in form of liquid extracts prepared from the ephedrine, as when administered by injection.

Gastro-intestinal tract—The movements of the small intestines are markedly inhibited by intravenous injection of pseudo-ephedrine in intact animals and of the isolated intestine when it is added to the perfusing fluid. The tone of the intestinal muscles is decreased and peristaltic movements are completely stopped. Although the movements soon reappear the rhythm and force of contraction remain depressed for a long time.

The action of pseudo-ephedrine on gastric and intestinal secretions is still under investigation. In our clinical trials, however, the gastro-intestinal disturbances sometimes observed after ephedrine were generally absent after pseudo-ephedrine. On the whole there is little difference between the action of ephedrine and pseudo-ephedrine on the gastro-intestinal tract.

Circulatory system—Heart The action of pseudo-ephedrine from the Indian varieties of ephedra on the heart was fully investigated and results have been published in a separate paper (*Ind Jour Med Res*, XVI, 3). The main conclusions are that pseudo-ephedrine stimulates both the inhibitory and the accelerator mechanisms of the heart and has a stimulating influence on the myocardium. The rise of blood-pressure is not so great as in case of ephedrine and is only partly due to sympathetic stimulation as it is still produced when the sympathetics are paralysed with ergotoxine. The occurrence of the rise after the vaso-motor fibres are paralysed show that the alkaloid stimulates the unstriped muscle fibres of the blood vessels. There is also reason to believe that the cardiac muscle is also stimulated. The irritability of the heart muscle, as tested by stimulating the surface of the ventricle by induced tetanizing shocks from a Du Bois Raymond inductorium, was found to be increased by this alkaloid. This view is supported by the fact that auricular fibrillations are sometimes produced after injection of even small doses of pseudo-ephedrine.

Experiments with perfusion of the isolated heart shows that pseudo-ephedrine produces well marked augmentation of the amplitude of contractions in such dilutions as 1 in 200,000. An increase in frequency of heart beat is produced even after administration of ergotoxine and nicotinic acid, pointing in all probability to its direct action on the heart muscle. The main difference between the two alkaloids thus is that while ephedrine depresses the heart muscle pseudo-ephedrine stimulates it. The stimulant effect of the drug on the heart muscle, however, is not so evident when there is high peripheral resistance. When this is no longer present, as is the case when the vaso-motor nerves are paralysed by ergotoxine, the action of the drug becomes quite apparent.

Blood vessels—Pseudo-ephedrine produces constriction of the arterioles in very much the same way, though not to the same extent, as ephedrine. It appears probable that it has some effect on the musculature of the blood vessels which ephedrine does not possess. This is shown by the fact that perfusion of arteries such as superior mesenteric in animals who have received paralysing doses of ergotoxine, showed a definite contraction of the blood vessels. It has been observed by some workers that pseudo-ephedrine produces a constriction of the blood vessels of the splanchnic area while it causes a dilatation of the peripheral vessels. In order to determine the action of the alkaloid separately on the two areas two sets of experiments were performed. In one series all the main vessels of the splanchnic area were tied so that only the peripheral vessels were left to be acted upon by the alkaloid. An injection of pseudo-ephedrine still produces a rise of blood-pressure. After exclusion of the peripheral vessels by ligaturing two femoral and the branchial arteries the pressor response was still produced. These experiments show that pseudo-ephedrine has the same action on the blood vessels of the splanchnic area as those of the peripheral area. The volume of spleen shows a well marked constriction after the

alkaloid but the volumes of the other abdominal organs do not show the same marked decrease in size. The intestine either shows a very slight increase or no effect at all, the kidney volume on the other hand shows a well marked increase showing that the vessels are dilated, the initial momentary contraction, present in the case of ephedrine, is absent. Most probably the effect on the kidney is secondary to the general rise in the systemic blood-pressure.

The pulmonary pressure shows a marked rise, the action resembling that of adrenaline. This is one of the most constant effects of the drug. The rise appears to be due to contraction of the branch of pulmonary artery which also relieves the turgescence of the mucous membrane.

Respiratory system—Pseudo-ephedrine produces a well marked dilation of the bronchioles. It not only dilates the bronchioles in decerebrated animals but quickly relieves the spasm artificially produced by such drugs as pilocarpine. Its value in asthmatic conditions can, therefore, be readily appreciated.

The marked shrinkage of nasal mucous membrane produced by ephedrine is also present with pseudo-ephedrine. Both the broncho-dilator and contracting action of this alkaloid on the mucous membrane in the nose are nearly as marked as ephedrine.

Genito-urinary system—*Uterus*. The movements of the isolated virgin uterus of a cat are inhibited by pseudo-ephedrine in concentrations about 1 in 75,000. The pregnant uterus of the same animal does not show any marked change in tone or movements in such concentrations. The action on rabbits virgin uterus is also not very marked, there being little or no movements of the organ with concentrations varying between 1 in 150,000 and 1 in 50,000. The virgin uterus of a cat in intact animals shows relaxations after 2 mgm doses.

Kidney—Pseudo-ephedrine given intravenously caused a well marked increase of the flow of urinary secretion which runs parallel with the duration of the vaso-pressor action. Subsequent injection of the drug produces a smaller and smaller rise of blood-pressure but the diuretic effect persists though it is not quite so marked. It appears, therefore, that two factors are concerned in the production of diuretic action of this alkaloid namely (1) the vaso-pressor action on the general circulation, and (2) some direct action on the renal cells. This is shown by the fact that acceleration of flow of urine still occurs, though to a lesser degree, when the extension of the kidney is prevented by encasing it in plaster of paris.

Nervous system—That the effects produced by an injection of pseudo-ephedrine are not central but peripheral is shown by the fact that exactly similar results are obtained after destroying the brain and the spinal cord of animals. Pseudo-ephedrine does not appear to have any effect on the higher cerebral centre. The medullary centre such as vaso-motor centre is not affected as is shown by the perfusion aspects, the respiratory centre is also not affected as shown by Thomas and Franks (1928) experiments with strips of diaphragm.

Injection of the alkaloid does not produce any marked change in the movements of the strip showing that respiratory centre is not affected by the drug

Action on secretions—The salivary and sweat secretions are not affected by this alkaloid

Differences in the action of ephedrine and pseudo-ephedrine

From the experimental data we have collected, it will be seen that the action of pseudo-ephedrine closely resembles that of ephedrine. Both the alkaloids pass through the liver unchanged and produce their usual effects whether injected into one of the mesenteric veins or into a systemic vein. They are both rapidly absorbed from the gastro-intestinal tract and their inhibiting effect on the musculature of the gut is about equal. Both the alkaloids produce a contraction of the blood vessels and a well marked rise of the blood-pressure. The vaso-pressor effect is much stronger in case of ephedrine which acts almost entirely on the vaso-motor nerve-endings, while pseudo-ephedrine has been shown to have some action on the musculature of the vessels as well. The rise of pressure is also less marked in the pulmonary and portal areas with pseudo-ephedrine. Its dilator action on the bronchioles as well as its contracting action on the mucous membrane of the nose do not essentially differ in its potency from that of ephedrine. The effect of the two alkaloids on the kidney is to produce a dilatation of the blood vessels and increase of the kidney volume, but the initial momentary constriction produced by ephedrine is absent in case of pseudo-ephedrine, the diuretic effect is much more marked in case of the latter alkaloid. The action of the two alkaloids on the voluntary and involuntary muscles appears to be about equal.

THERAPEUTIC USES OF INDIAN EPHEDRA

It has been already remarked that the pseudo-ephedrine content of the Indian species of ephedra is high. The yield of ephedrine from various varieties in many cases does not exceed 50 per cent of the total alkaloids and often is considerably less. The price of the alkaloid is now about Rs 600 per pound and even at that sufficient quantities are not available. Some of the Indian varieties contain much larger quantities of pseudo-ephedrine than ephedrine. In view of these facts we tried to see how far it was possible to substitute pseudo-ephedrine for ephedrine in therapeutics.

Ephedrine and pseudo-ephedrine in the treatment of asthma

From the time the sympathomimetic action of ephedrine was discovered this alkaloid has been very extensively used in the treatment of asthma. The relief afforded by it though not quite so instantaneous as adrenaline, is quick and certain besides it can be taken by the mouth and need not be given by injection. It has, therefore, been used indiscriminately in large majority of cases.

with sometimes untoward results. We have known patients who have been in the habit of taking half a grain of the alkaloid twice a day for many months. In our asthma clinic at the Calcutta School of Tropical Medicine, our experience with the use of this alkaloid in the treatment of this symptom-complex has not been altogether satisfactory. It undoubtedly controls the paroxysms and relieves the symptoms in quarter of an hour to half an hour but it is likely to produce unpleasant side effects. In some patients acute pain in the cardiac region lasting for 10 to 20 minutes has been observed and a feeling of distress in the præcordium is not an uncommon symptom in a large number of patients using the drug, due to hypertension produced by stimulation of the vaso-motor nerve-endings. Some patients get palpitation, flushing of the skin and tingling and numbness of the extremities, tachycardia and fainting fits may be produced. Patients suffering from inflammatory conditions of the skin frequently get exacerbation after its use and quiescent conditions may become acutely active. Those suffering from organic disease of the heart, especially of the myocardium, get decompensation, probably due to depressant action on the heart muscle by excessive dosage.

Besides this the stimulating action of the alkaloid on the sympathetic is liable to produce persistent constipation, which aggravates certain types of asthmas. Loss of appetite frequently occurs and digestive disturbances are not infrequent accompaniments. This drug has not been sufficiently long in use for us to know all its untoward and toxic effect, but they undoubtedly do exist. Caution is, therefore, recommended in its use, especially for prolonged periods in the treatment of such a symptom-complex. Often the relief afforded is of short duration and there is temptation of repeating the drug. Its routine use in controlling the paroxysms without investigating the cause is to be strongly deprecated.

We have already pointed out that the pressor action of pseudo-ephedrine is much less powerful than that of ephedrine but its broncho-dilator action appears to be quite as marked. The contraction of the branches of the pulmonary artery relieves the turgescence of the mucous membrane and this with the well marked dilatation of the bronchioles helps in relieving the paroxysm. One of us (R. N. C.) has tried pseudo-ephedrine in the treatment of this condition with excellent results. Within 15 minutes to half an hour of oral administration of $\frac{1}{2}$ gram of the alkaloid, the feeling of tightness round the chest is relieved and the patient's breathing becomes normal. A similar dose taken when the premonitions of an attack are felt generally stops the paroxysm. The effect in fact is just as rapid as that of ephedrine. Although we have not tried it on a sufficiently large scale and for long enough periods, the results so far have been encouraging, and the side effects produced are not so unpleasant. If use of this alkaloid is extended in the treatment of asthma and other conditions in which ephedrine is being used, not only will the cost of treatment be reduced but it may be possible to avoid the unpleasant side effects of the latter drug.

Alcoholic extract and tincture prepared from Indian ephedra

An extract prepared from *E Gerardiana* and *E intermedia* first introduced by the senior author has now been in use for nearly three years. It is prepared by exhausting the dried powdered twigs of the plant with 90 per cent alcohol, sufficient water being then added to make the strength of alcohol about 45 per cent. Five c.c. of the extract should contain $\frac{1}{2}$ grain of the total alkaloids. This extract can be used either by itself or in combination with asthma mixtures and is very effective in controlling asthmatic paroxysms. It is considerably cheaper than the purified alkaloids and brings the use of this drug within the means of poor people. A weaker tincture is also on the market now.

Ephedrine and pseudo-ephedrine as cardiac stimulants

The stimulant action of these alkaloids on the blood-pressure is well known and for this reason they have been used as cardiac stimulants. We have already pointed out that while ephedrine, especially in large doses, has a depressant action on the myocardium, pseudo-ephedrine, on the other hand, has the opposite stimulant action on the heart muscle. Besides its action on the vaso-motor nerve-endings the latter alkaloid also stimulates the muscle fibres of the arterioles. The senior author has, therefore, tried an extract from ephedra which contains both ephedrine and pseudo-ephedrine (more of the latter) as a cardiac stimulant with encouraging results. This produced a well marked beneficial effect when administered to patients in whom the action of the heart was weak and compensation was failing. Our observations on a number of patients showed that there was a definite rise of blood-pressure amounting to 10 to 20 mm of mercury, after $\frac{1}{2}$ to 1 dram doses 2 or 3 times a day. Marked diuresis was produced in those patients in whom the function of the kidneys was disturbed from inefficient circulation.

Epidemic dropsy—As is well known the heart is seriously affected in this condition giving rise to such subjective symptoms as dyspnoea, palpitation, præcordial pain and even cardiac asthma. The rate of heart beat is accelerated from the very beginning of the disease. The first sound at the apex becomes short and sharp, and later it becomes muffled, often the first sound is reduplicated. Later a systolic murmur may be present at the apex due to dilatation of the heart producing mitral incompetence and sometimes a hæmic murmur is also audible at the pulmonary base. A presystolic murmur may be heard. In such cases digitalis gives unsatisfactory results, in fact some of the patients actually become worse. A number of other cardiac stimulants proved ineffective. In cases of left heart failure, the tincture of ephedra proved very effective. The patient felt relieved and the symptoms disappeared.

Other cardiac conditions—The tincture of ephedra is also an excellent cardiac stimulant in toxic conditions of the heart produced by such infections as pneumonia diphtheria, etc. Lieut-Colonel Vere Hodge, R.M.S., tried the tincture in $\frac{1}{2}$ dram doses 3 or 4 times daily with excellent results.

SUMMARY AND CONCLUSIONS

1 A suitable method of assay of ephedra has been developed. The alkalinization with ammonia and the action of chloroform on ephedrine have been fully discussed.

2 Localities in which ephedras grow in India have been worked out and a map showing the distribution of the important species is attached.

3 Ephedras from many different localities in India have been assayed. It has been found that *E. foliata* has no ephedrine, *E. intermedia* has about 10 per cent of, *E. Gerardiana* and *E. nebrodensis* have about 70 per cent of ephedrine in the total alkaloid.

The richest sample of *E. intermedia* analysed contained 2.33 per cent total alkaloid of which 0.38 per cent was ephedrine and 1.8 per cent pseudo-ephedrine. This sample was obtained from Chini Range, Bashahr Division. *E. intermedia* from Chini Range may be used as a source of supply of pseudo-ephedrine.

The richest sample of *E. Gerardiana* was found in Kagan (N-W P) and this contained 2.15 per cent of total alkaloids of which 1.53 was ephedrine.

The richest sample of *E. nebrodensis* was found in Lahoul and this contained 2.79 per cent of total alkaloids and 1.93 per cent of ephedrine.

4 The ephedrine content in Indian ephedras does not increase with the altitude of the locality where the drug grows.

5 The rainfall of the locality influences the alkaloidal content of Indian ephedras. The greater the annual rainfall the smaller is the alkaloidal content.

6 The ephedrine content in the plant varies with the time of the year. It is usually lowest during the wet months and highest in the autumn.

7 The alkaloidal content in the drug does not decrease appreciably on storage.

8 A cheap method of large scale extraction with dilute hydrochloric acid is described.

9 The action of chloroform and related compounds on natural and synthetic ephedrine is described.

10 Ephedrine has been found to occur in some of the other Indian medicinal plants, e.g., *Sida cordifolia* which contains it to the extent of 0.085 per cent in the whole plant and 0.32 per cent or more in the seed.

11 Ephedrine and pseudo-ephedrine from the Indian varieties of ephedra have a stimulant action both on the sympathetic and vagus nerve-endings of the heart. Ephedrine stimulates the sympathetic ganglia also.

12 Both ephedrine and pseudo-ephedrine have a pressor action, the former being more powerful than the latter, the rise of blood-pressure is produced by direct stimulation of the vaso-motor nerve-endings. The persistent rise seen after paralysis of the vaso-motor nerves in the case of pseudo-ephedrine is due to the increase of cardiac output and also stimulation of the muscles of the blood vessels.

13 Pseudo-ephedrine stimulates the myocardium while ephedrine, especially in large doses, depresses it

14 Dilator action of pseudo-ephedrine on the bronchioles is nearly as marked as that of ephedrine and so is its constricting action on the mucous membrane of the nose. Its value in asthmatic conditions can, therefore, be readily appreciated. The alkaloid has no action on the respiratory centre.

15 Pseudo-ephedrine has a more powerful action as a diuretic than ephedrine. The action is mainly vascular, the vessels of the kidney being dilated and the volume of the organ increased.

16 Both ephedrine and pseudo-ephedrine are useful in asthma and as cardiac stimulants. Ephedrine, however, gives rise to unpleasant side effects, especially in the treatment of asthma. Pseudo-ephedrine is just as effective in relieving paroxysms and therefore can be substituted for ephedrine. On account of its marked diuretic properties it would be valuable as a cardiac tonic where oedema is present.

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NOTES ON SOME INDIAN MOSQUITOES OF THE SUBGENUS *STEGOMYIA*, WITH DESCRIPTIONS OF NEW SPECIES

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[Received for publication, March 6, 1931]

It has recently been found that, up to now, several Indian species of *Aedes* (*Stegomyia*) have been confused with *albopictus* (Skuse). One of these appears to be *Aedes* (*S*) *pseudalbopictus* described by Boel from Cochin China, two others appear to be new and are described below. These three species resemble *Aedes* (*S*) *albopictus* very closely indeed, differing chiefly in the structure of the male genitalia. At present it has not been possible to correlate the females with the males of these different species, but later it may be possible to do so by a close study of the larvæ, and by breeding series of adults from single females.

The identity of *Aedes* (*S*) *albopictus* is of some medical importance as it has been shown experimentally to be capable of transmitting both dengue fever and yellow fever (Simmons and others, 1930) (Dinger and others, 1929).

Some notes are given on this species below, and on *Aedes* (*S*) *flavopictus* Yun. The latter, which also resembles *Aedes* (*S*) *albopictus* very closely, was originally described from Japan, but has been recorded from India by me previously.

Another species of the same subgenus which resembles *Aedes* (*S*) *annandalei* Theo. and *Aedes* (*S*) *mediopunctatus* Theo. very closely in markings, but differing remarkably in the structure of the male hypopygium, is described also under the name *Aedes* (*S*) *pun.*, after Dr I. M. Pun, who collected the specimens in North Bengal.

Camera lucida drawings, all to the same scale, are reproduced on the accompanying plate (Plate XI), illustrating the structure of the male genitalia of the six species dealt with in this paper. It should be noted that the drawings have been made from stained preparations, and from separate parts

mounted flat after dissection of the hypopygium. The different parts cannot be seen to advantage, nor the corresponding structures in different species properly compared, unless the preparations are mounted in this way. I am much indebted to Mohamed Yusaf, Laboratory Assistant, for the trouble he has taken in making several hundreds of careful preparations for me.

***Aedes (Stegomyia) albopictus* (Skuse)**

Systematic references —

Culex albopictus Skuse, 1894, *Ind Mus Notes*, **3**, No 5, p 20 (♀ described from Bengal)

Stegomyia scutellaris (Walk.), Theobald, 1901, *Mon Cul*, **1**, pp 298-300 (♂ and ♀, fig 91, ♂ unguis Plate XIV, adult ♀)

Stegomyia scutellaris (Walk.), Leicester, 1908 *Cul Malaya*, pp 86-87 (♂ and ♀)

Stegomyia albopicta (Skuse), Edwards, 1917 *Bull Ent Res*, **7**, pp 209-210 (Fig ♂ hypop)

Stegomyia albopicta (Skuse), Barraud, 1923, *Ind Jour Med Res*, **10**, pp 779-780 (♂ and ♀, ♂ hypop Plate XLIII, fig 3, XLV, fig 15, XLVII, fig 23, LIV, figs 1-6)

Aedes (Stegomyia) albopictus (Skuse), Brug, 1924, *Vereen tot Bev d Geneesk Wetens in Ned Ind*, pp 41-43, (♂ and ♀, Figs markings thorax and abdomen)

Stegomyia albopicta (Skuse), Borel, 1928 *Arch des Inst Pasteur d'Indochine*, No 7, pp 83-85, (♂ and ♀, larva Plate III, figs ♂ hypop larva)

The name *scutellaris* Walker (*Culex*) was wrongly applied to this species by Theobald and Leicester. This name is now treated as a synonym of *Aedes (S) variegatus* Doleschall (*Culex*).

♂ in the specimens I have examined the palpi are longer than the proboscis by about half the length of the apical segment. This character may be variable as in Leicester's description it is mentioned that the palpi are longer than the proboscis by the length of the terminal segment.

♂ hypopygium 9th tergite (Fig 1) strongly produced in the middle into a blunt point (differing from all other Indian species in the group in this respect), a pair of well developed sublateral hairy lobes, coxite (side-piece) about 3 times the length of the greatest width (when separated and mounted flat), basal plaque on inner surface of coxite (Fig 11) with stem of moderate length and width, apical part flattened (when mounted separately) carrying some blade-like processes on the ventral (sternal) side, several of which are long and curved, there are also a number of hairs with bent tips, style (clasper) (Fig 13) comparatively rather short, of fairly even width throughout, and with the appendage arising very near the rounded tip.

- Distribution (checked by examination of male genitalia) —
 LOWER BURMA Tenasserim, 10-12 1 1922 (*Sharma*)
 UPPER BURMA Lashio, 23-24 viii 1913 (*T B Fletcher*)
 ANDAMAN ISLANDS vii 1926 (*G Covell*)
 ASSAM Gauhati, ix 1928 (*Sobha Ram coll*), Shillong, vi and viii 1922 (*Barraud*), Haflong and Dibrugarh, viii 1922 (*Barraud*)
 BENGAL (NORTH) Tirrihana and Manianbanne Tea Estates, and Sukna, viii 1928 (*Puri*), Tindharia (Darjeeling Rwy), viii 1928 (*Sobha Ram coll*)
 BENGAL (EAST) Chittagong, viii 1922 (*Barraud*)
 ORISSA Uluburu, Keonjhar State, 26 ix 1928 (*Senior White*)
 BIHAR Pusa, 178 specimens examined from the collection of the Agricultural Research Institute Occurs throughout the year but more abundantly during the rainy season
 PUNJAB Kainai, viii 1930 (*Barraud*)
 WESTERN HIMALAYAS Kasauli, vi 1923 and vii 1924 (*Barraud*)
 DELHI PROVINCE Delhi (specimens in the M S I collection)
 KONKAN Bombay Harbour, Elephanta Island, 6 vii 1921 (*Barraud*)
 Trombay, vii 1921 (*Barraud*), Hog Island, 9 vii 1921 (*Barraud*)
 BOMBAY DECCAN Belgaum, 1 viii 1921 (*Barraud*), Nagargali, 13 viii 1921 (*Barraud*)
 MALABAR Coorg, vi 1927 (*J D Barly*), Malabar Coast, 1915 (*Khazan Chand*)
 MADRAS Nilgiri Hills, ix 1915 (*Khazan Chand*), Anamali Hills, 4-8 viii 1928 (*Shaffi*)
 CEYLON Suduganga Estate, Matale, 5 v 1919 (*Senior White*)

***Aedes (Stegomyia) pseudalbopictus* Borel**

Stegomyia pseudalbopictus Borel, 1928 *Arch des Inst Pasteur d'Indochine*, No 7, pp 85-87 (♂ and ♀ larva Plate IV, figs ♂ hypop larva)

There do not appear to be any constant differences in markings by which the males of this species may be distinguished from those of *Aedes (S) albo-pictus*, but there are very marked differences in the structure of the genitalia. The palpi are longer than the proboscis by rather more than half the length of the apical segment.

♂ hypopygium 9th tergite (Fig 2) nearly flat in the middle, a pair of large sublateral hairy lobes, coxite from 3.2 to 3.4 times the length of the greatest width (when separated and mounted flat), basal plaque on inner surface of coxite (Fig 7) unusually long and narrow with one strong spine-like process and numerous hairs distal to it, several of which, at the apex, are clubbed, style (Fig 16) comparatively long and slender, appendage stout and arising some distance from the tip which is pointed

Distribution—Type locality, Terres Rouges, Cochin China, larvæ in bamboo stumps during rainy season

Males have been examined from the following places in India —

BENGAL (NORTH) Marianbairie Tea Estate, near Sukna, viii 1928 (*Puri*), Sukna, viii 1928 (*Puri*)

BOMBAY DECCAN Nagargali, 13 viii 1921 (*Barraud*)

***Aedes (Stegomyia) flavopictus* Yam**

Aedes flavopictus Yamada, 1921 *Annotationes Zoologicae Japonenses*, 10, Article 6, pp 52-54 (♂ and ♀, Fig ♂ hypop)

Stegomyia flavopicta Yam, Barraud, *Ind Jour Med Res*, 11, 1923, pp 225-6 (♂ and ♀, Plate XIX, figs ♂ hypop)

As stated in the original description and in my paper quoted above this species may be distinguished from *Aedes (S) albopictus* by the colour of the mesonotal markings, which, except for the median silvery stripe, are yellowish. I find however that in many specimens the yellowish tinge is very faint or even wanting, possibly due to fading, and it is therefore almost impossible to distinguish these from *albopictus*. The males may however be easily separated by examination of the genitalia. The palpi are comparatively shorter than in *albopictus* being only very slightly longer than the proboscis.

♂ hypopygium 9th tergite (Fig 3) moderately wide, the middle part produced into a rounded lobe, sublateral lobes not well developed and with few hairs, basal plaque on inner surface of coxite (Fig 9) with narrow stem and wide apical part carrying flattened, slightly clubbed, processes on the sternal aspect, and hairs all about the same length, style (Fig 14) very similar to that of *Aedes (S) albopictus*, but both this and the appendage rather longer, the latter arises a short distance from the tip of the style.

Distribution—Type locality, Shiba, Tokyo, Japan, 20 iv 1916 (*Yamada*), also occurs in Honshu and Hokkaido, Japan, and Korea.

Males have been examined from the following places in India —

ASSAM Shillong, 5,000 ft vi 1922 (*Barraud*)

WESTERN HIMALAYAS Muirce, 7,000 ft 1922 (*Gill*), Kasauli and Kiol Mountain, near Solon, 6,000 to 7,000 ft commonly breeding in tree-holes during the monsoon (*Barraud*)

MALABAR Coorg, Mercara, vi 1927 (*J D Barly*)

***Aedes (Stegomyia) novalbopictus* sp n**

I have been unable to discover any constant differences in markings between this species and *Aedes (S) albopictus*, and the published descriptions of the latter (quoted under that species above) would appear to apply equally well to this species also, except in certain structural points mentioned below.

♂ the palpi are only about the length of the proboscis, but may be very slightly longer or shorter than that organ.

♂ hypopygium 9th tergite (Fig 5) with the middle part forming a wide slightly rounded lobe, with sometimes a very small rounded projection in the centre of the apical margin (when the tergite is separated and mounted flat this projection is not usually seen), sublateral hairy lobes fairly well developed, basal plaque on inner surface of coxite (Fig 8) with broad stem, numerous hairs of varying lengths, many with bent tips, arising from the apex, and a row of slender flattened spines along the sternal side, these spines often appear sharply pointed but are actually slightly expanded just below the tips, style (Fig 18) moderately long and of even width throughout, the appendage arising very near the tip

Distribution—Type male from Pusa, Bihar, 27 vii 1916 (S K S) now in the Malaria Survey of India collection, Kasauli. Out of 188 males of '*albopictus*' examined from the collection of the Agricultural Research Institute, Pusa, 10 proved to be the new species here described. Particulars of the other males which have been examined are as follows —

BIHAR Pusa, 16 ii 1908 (*H N S*), 14 iii 1913 (*H N S*), 8 iv 1913 (*Md S*), 10 vii 1913 (*Md S*), 2 vii 1914 (*Md S*), 15 iv 1921 (*Shafi*), 26 v 1921 (*Shafi*) Pusa, bred from tree-hole material, ii 1931 (*Barraud*)

ORISSA Ranchi, 21 viii 1922 (*T B Fletcher*)

WESTERN HIMALAYAS (Foothills) Koti, near Kasauli, bred from tree-hole material, iii 1931 (*Barraud*)

KONKAN Bombay Harbour, Elephanta Island and Trombay, vii 1921 (*Barraud*)

BOMBAY DECCAN Belgaum, 1 viii 1921 (*Barraud*)

***Aedes (Stegomyia) subalbopictus* sp. n**

The male of this species resembles that sex of *Aedes (S) albopictus* very closely in the ornamentation of the head, thorax, and legs, but differs (in the only specimen known) in having the dorsum of the abdomen entirely dark without any indication of basal silvery bands, there are small basal lateral silvery patches on the tergites. The palpi are about the same length as the proboscis.

♂ hypopygium this shows a number of differences in structure from that of *Aedes (S) albopictus*. Ninth tergite (Fig 4) with the middle part forming a wide curved lobe, sublateral lobes small, not projecting, each with about 4 hairs, coxite rather more than 3 times the length of the greatest width, basal plaque on inner surface of coxite (Fig 10) with a long moderately broad stem, the apex carrying a number of slightly curved blade-like processes and hairs, the majority of which have hooked tips, the hairs and processes all about the same length, style (Fig 15) unusually long, the tip pointed, the appendage arising some little distance from it.

Type male (unique) in the Malaria Survey of India collection, Kasauli, from Belgaum, Bombay Deccan, viii 1921 (*Barnaud*)

***Aedes (Stegomyia) purii* sp. n**

Only two specimens of this species are known, both males and in poor condition. As far as can be seen they resemble *Aedes (S) annandalei* Theo, and *Aedes (S) mediopunctatus* Theo, very closely in ornamentation. It is not possible to say whether they differ from one or both of these species in the markings of the mesonotum as both specimens are very much denuded of scales. A description is given below but it should be noted that this description would equally apply to poor specimens of *Aedes (S) annandalei*, except as regards the structure of the hypopygium. This in several respects is remarkably different from that of any other Indian species.

♂ head mainly flat scaled, a collection of dark upright scales on nape, a triangular patch of white scales in the middle of vertex in front, continued between eyes, black scales surrounding the white patch, white scales forming lateral patches, black scales at sides followed by white scales again low down, white scales on inner side of torus and beneath, antennal plumes dark brown, palpi a little longer than proboscis with two conspicuous white rings, one near the base and one in middle of long segment, some white scales at bases of last two segments chiefly beneath, proboscis black. *Thorax* mesonotum and scutellum in both specimens nearly completely denuded, some flat white scales remain over wing roots and some flat black scales on posterior part of disc, a few rather narrow white scales on front margin, flat white scales on lateral lobes of scutellum, broad black flat scales on mid lobe, flat white scales on anterior pronotal lobes and on lower part of posterior pronotal lobes (upper part bare or denuded), broad flat white scales forming a patch between anterior spiracle and wing root, another large patch on upper part of mesepimeron. *Wings* as usual in the subgenus, with dark brown scales on veins. *Legs* fore femur dark anteriorly, pale posteriorly on basal half, mid femur dark anteriorly with some pale scales at tip, no white spot, dark posteriorly except ventrally where it is narrowly pale on basal half or rather more, hind femur entirely pale on rather more than basal half, white scales forming knee spot, tibiae brownish-black, fore tarsi dark except for a narrow white ring at base of segment 1, mid tarsi similarly marked but with a few pale scales at base of segment 2 also, hind tarsi with wider white rings at bases of segments 1 and 2, segment 3 dark, 4 mainly white except for a narrow black ring at tip, 5 dark. *Abdomen* partially denuded in both specimens, dorsum apparently mainly brownish-black with lateral basal white patches, indications of narrow basal white bands in one specimen, sternites black with basal white bands.

♂ hypopygium 9th tergite (Fig 6) very narrow and concave in the middle, sublateral lobes small, each with about 3 hairs, basal plaque on inner surface of coxite (Fig 12) unusually long with very numerous hairs and 3 strong

haipago-like processes near base, style (Fig 17) of moderate length and width, rather swollen towards apex and tapering sharply to tip, appendage long and stout, arising some distance from tip of style

Two co-type ♂♂ in the Malaria Survey of India collection, from Marianbaine Tea Estate, near Sukna, North Bengal, caught in jungle, viii 1928 (Dr I M Puri)

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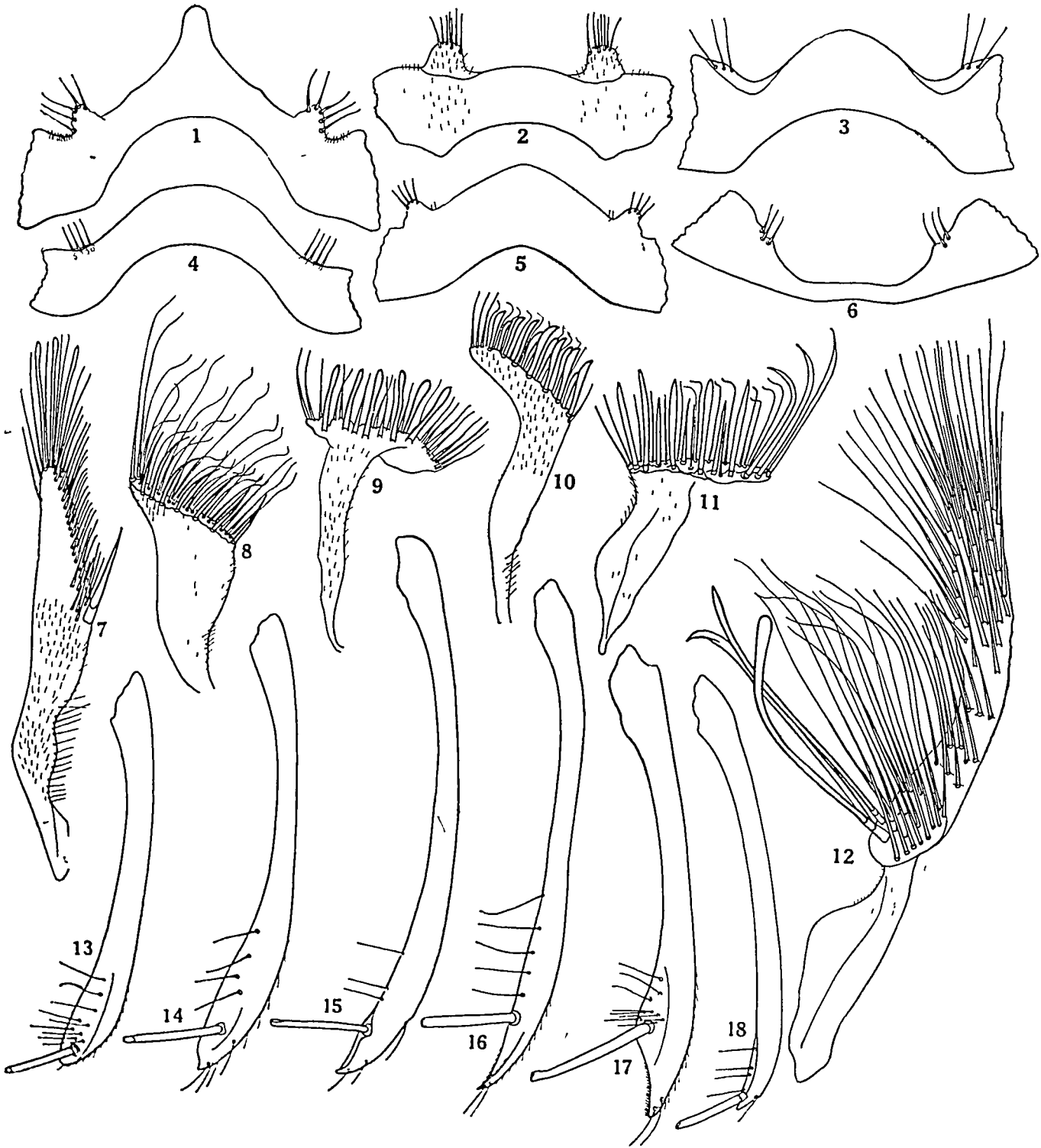
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EXPLANATION OF PLATE XI

Camera lucida drawings, all to the same scale, illustrating parts of the hypopygia of six species of *Aedes* (*Stegomyia*), drawings made from stained preparations of separate parts mounted flat

Fig 1	<i>Aedes</i> (<i>Stegomyia</i>)	<i>albopictus</i> (Skuse)	Ninth tergite
" 2	"	" <i>pseudalbopictus</i> Borel	Ninth tergite
" 3	"	" <i>flavopictus</i> Yam	Ninth tergite
" 4	"	" <i>subalbopictus</i> sp n	Ninth tergite
" 5	"	" <i>novalbopictus</i> sp n	Ninth tergite
" 6	"	" <i>purn</i> sp n	Ninth tergite
" 7	"	" <i>pseudalbopictus</i> Borel	Basal plaque from anal surface of coxite
" 8	"	" <i>novalbopictus</i> sp n	Basal plaque from anal surface of coxite
" 9	"	" <i>flavopictus</i> Yam	Basal plaque from anal surface of coxite
" 10	"	" <i>subalbopictus</i> sp n	Basal plaque from anal surface of coxite
" 11	"	" <i>albopictus</i> (Skuse)	Basal plaque from anal surface of coxite
" 12	"	" <i>purn</i> sp n	Basal plaque from anal surface of coxite
" 13	"	" <i>albopictus</i> (Skuse)	Style and appendage
" 14	"	" <i>flavopictus</i> Yam	Style and appendage
" 15	"	" <i>subalbopictus</i> sp n	Style and appendage
" 16	"	" <i>pseudalbopictus</i> Borel	Style and appendage
" 17	"	" <i>purn</i> sp n	Style and appendage
" 18	"	" <i>novalbopictus</i> sp n	Style and appendage

PLATE XI



BASAL METABOLISM OF THE PRISONERS OF THE DISTRICT JAIL, LUCKNOW (UNITED PROVINCES OF AGRA AND OUDH)

BY

LIEUTENANT NIAN T DHAN BANERJI, M B, B S, A I R O

[Received for publication, March 20, 1931]

Note—The entire work, which is original, has been carried out by me under the direction of the Head of the Department of Physiology, King George's Medical College, Lucknow. The expenses were met entirely from the Capt. Kunwar Indrajit Singh, M C, I M S, Research Fund of the King George's Medical College, Lucknow University.

The apparatus which has been used throughout the experiments is the 'British Benedict portable metabolism apparatus closed circuit type' (see Plate XII).

The technique has been followed as indicated in the leaflet M 16 (*Lancet*, 1921, 1924).

The total number of individuals investigated was 145, including all classes, i.e., Hindus and Mohamedans. The total number of observations made was 305.

Constants—Certain prisoners of superior intelligence had their basal metabolism frequently determined. By this means a check was kept on the apparatus used and also seasonal variations noted. In addition they were exceedingly useful in demonstrating to the less intelligent the fact that the determinations were not harmful to the experimental subject.

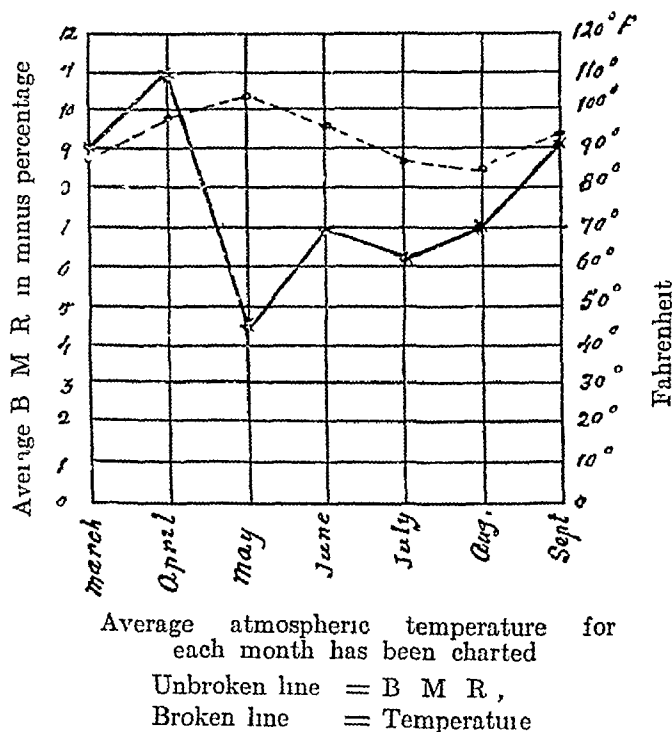
Results—I first give in the protocol the actual result of 100 different determinations. They show a divergence from English and American standards of 6.9 per cent (see Appendix).

I found in Lucknow that basal metabolism was subject to variation as was indeed to be expected because it has already been shown in Europe and America that basal metabolism is higher in winter than in summer.

I found, however, that in the Lucknow (United Provinces) jail the basal metabolism did not vary in the direction to be expected if external temperature were the primary factor determining variation, as may be gathered from Graph 1

GRAPH 1

1929



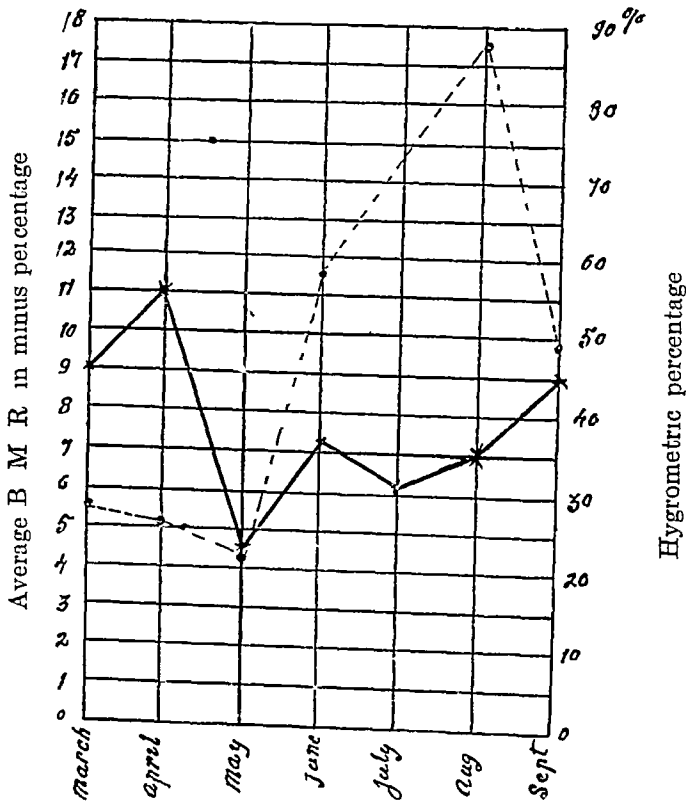
In the month of March the average atmospheric temperature in Lucknow was about 87°F and the average B M R for the month shows —9.2 per cent. In the next month the temperature average to 95.2° but the B M R falls to —11.0 per cent. In May the temperature rises to 102.5° and the B M R shows the maximum rise. In June the temperature falls to 94.9°F and B M R falls to about —7.4 per cent, but this fall is not so sharp as one would expect considering the fact that the average temperature in both the months of April and June are nearly the same. July records a further fall in temperature but B M R rises compared with June. The month of August records 85.4°F and B M R again falls when compared with the previous month. In September temperature rises but the B M R instead of rising shows a greater fall than any of the previous months excepting the month of April. Therefore we conclude that the temperature curve and the B M R curve are not parallel.

Graph 2 shows the B M R and atmospheric humidity curve. In March the average atmospheric humidity comes to 27.5 per cent and the B M R shows an average of —9.2 per cent. Next month the B M R shows the

greatest fall while the humidity averages to 25.6 per cent. In the month of May the humidity shows the greatest fall and the B M R rises. In June humidity rises to 57.0 per cent and the B M R falls. July records a still greater percentage of humidity but B M R slightly rises. The month of August records the highest humidity percentage and the B M R falls when compared with the previous month. In September the humidity comes about the June standard but the B M R shows a still further fall when compared

GRAPH 2

1929



Average atmospheric humidity for each month has been charted

Unbroken line = B M R,

Broken line = Hygrometric curve

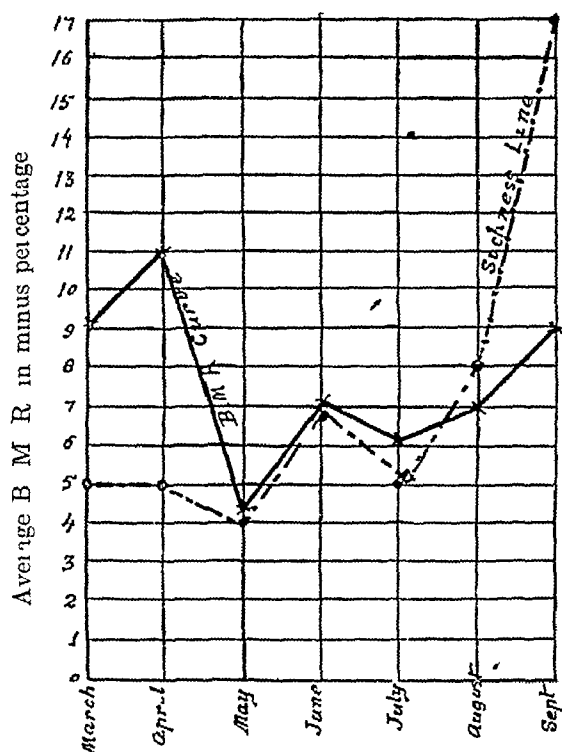
with the previous month of August. Probably the maximum effect of the humid wave of August was reflected on the B M R in September. From the last days of May humidity rose in the succeeding months of June, July and August. The result of this long spell of humidity was a definite fall in the B M R except for a slight rise in July. We observe from Graph 2 that the B M R from month to month, except for the month of April, follows more closely the atmospheric humidity curve rather than the atmospheric temperature curve.

The results show that atmospheric humidity is a highly important determinant of basal metabolism which tends to fall as humidity rises and vice versa

In Graph 3 we plot together sickness ratio and B M R and find a tendency for them to run parallel. This may indicate that factors which depress basal metabolism are also favourable to the development of the bacterial enemies of man. But it may also be interpreted to indicate that the individual of

GRAPH 3

1929



Sickness Line = Direct reading of sickness per 700 of constant population along the ordinate

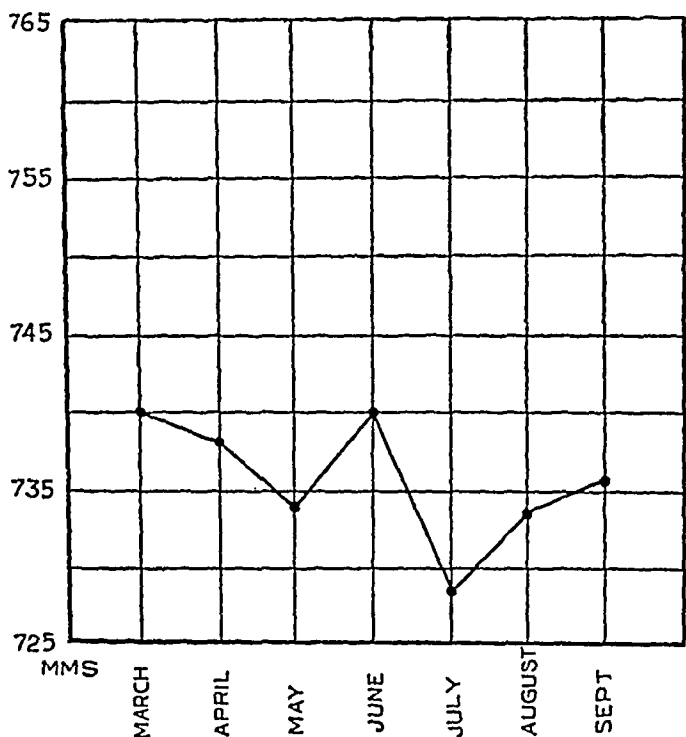
B M R Line = Direct reading in percentage along the ordinate

low B M R is a favourable soil for these enemies to develop in September, for instance, is one of the least healthy months of the year in Lucknow and, as the experiments show, is a month of low B M R. Hence, while the bacterial and insect enemies of man may find this a favourable month for their own development, it may be also that the low B M R of the human organism makes it a favourable soil.

Barometric pressure—Graph 4 does not bring out any fact very definitely, unless it be taken along with the temperature charts.

But we may cautiously say that as the pressure of the barometer falls, metabolism probably rises and vice versa. For example, in the month of May, according to Graph 1 the prisoner is at his best, while the pressure of the atmosphere at this period is low. When in April and September the prisoner is very low from the metabolic point of view we find the barometric pressure is higher than what it was in May. This is what we can guardedly interpret from the graphs.

GRAPH 4



Barometric pressure Average for each month has been charted for 1929 March to September

Scale, 1 div = 10 mms

Since the conclusion of the present work Mukerji and Gupta have published the results of their work on the basal metabolism of the Bengalis (*Ind Jour Med Res*, Jan 1931). They have found that the B M R of the Bengalis (males) is —13.3 per cent in Bengal. While Sokhey (1929) working in Bombay records a B M R of —9.0 per cent. If we add 7 per cent to the results of Miss E D Mason, Madras, we get —16.6 per cent as the B M R of the Madras males in Madras. M Ocampo, N Cordero and I Concepcion (*Jour Nutrition*, 1930, 3, 237) have worked out the basal metabolism rate of the Filipinos males and females respectively at 7.8 and 9.3 per cent lower than the Aub Du Bois standards (*Phy Abs*, Feb 1931). These figures are in accord with the results of the present research which show the great importance of humidity on basal metabolism. Calcutta, Bombay and Madras are towns

with a highly humid atmosphere and anyone acclimatized to any one of these places would be expected, according to my results, to show a lower basal metabolism than an individual living in a warmer, though drier, climate. These facts lead us to the logical conclusion that the basal metabolism rate of the Punjabees in the Punjab and of the populace of the N-W Frontier Provinces would be considerably higher than those of the United Provinces people, though at present we have no direct evidence on this.

Conclusions

1 That the average basal metabolic rate of the prisoners of the Lucknow (United Provinces) jail was found to be 6.9 per cent below the European standard.

2 That the high atmospheric temperature coupled with a high degree of humidity are probably the most important factors which go to lower basal metabolic rate of the Indians in India.

My thanks are due to Lieut-Colonel J. E. Clements, I.M.S., Inspector-General of Prisons, United Provinces of Agra and Oudh, to Major S. Salaamat Ullah, M.C., I.M.S., the Superintendent of the Jails, Lucknow, to Mr. I. U. Butt, Offg. Registrar, Lucknow University, and to my Professor of Physiology, Dr. W. Buiridge, without whose inspiration and guidance this paper would never have seen the light of the day.

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PLATE XII



Showing basal metabolism experiment in progress

APPENDIX

Male prisoners

Ages 20-45 years

Under-trials not included

Serial No	Weight (lb)	Height (inches)	Oxygen consumption Per cent
1	101	65 5	-9
2	116	66 5	-12 6
3	99	63	-6 5
4	119	66	-20 0
5	119	70	0 0
6	105	64 5	-1 5
7	106	65	-7 0
8	118	67	-13 0
9	106	65	-12 0
10	104	68 5	-20 7
11	130	68	-27 0
12	113	66	-16
13	101	66 5	-9
14	91	60 5	-20 0
15	115	65	-13
16	116	66	-12
17	123	64 5	-16
18	108	63 5	-11
19	107	62 5	-18
20	109	63	-2
21	92	64	-11
22	102	62 5	+1 6
23	119	65 5	-1 0
24	117	67 5	-12 5
25	99	66 5	-2
26	111	67 5	+14
27	114	65	-4 5

APPENDIX—*contd*

	Serial No	Weight (lb)	Height (inches)	Oxygen consumption Per cent
	28	126	69 5	0 0
	29	112	65 5	-8
	30	121	64 5	+1 0
	31	97	64 5	-20
	32	119	65	-8
	33	103	65 5	+13
	34	112	66 5	-15
	35	96	62	-17
	36	103	67	-4
	37	145	71	-22 3
	38	117	63 5	+19
	39	110	65	-20
	40	118	65 5	-14
	41	110	65 5	-6
	42	138	68	-0 9
	43	124	66 5	+1 0
	44	124	66 5	+0 5
	45	129	68	+6
	46	119	64 5	-9 4
	47	112	67	+8
	48	122	67	-17 7
	49	107	63 5	-2 5
	50	126	63 5	-3 8
	51	110	64 5	-2 0
	52	120	67	-14 4
	53	100	65 5	+0 5
	54	130	67	-12 9
	55	101	63 5	-0 5
	56	127	70 5	-8 4
	57	113	65 5	-0 5

APPENDIX—contd

Serial No	Weight (lb)	Height (inches)	Oxygen consumption Per cent
58	105	62.5	-1.5
59	101	61	+3.5
60	102	63	-7.9
61	129	65	-5
62	103	64	-5
63	127	69	-6.7
64	129	68.5	-20.0
65	100	61	-0.5
66	105	62	-15.9
67	109	70	-6.2
68	102	60.5	-22.1
69	112	66	-10.3
70	109	62.5	-3.6
71	96	61	+5
72	112	64	-9.1
73	99	63	-21.5
74	160	63.5	+2
75	110	65	-20.3
76	126	64.5	-5.7
77	94	63	+2.1
78	105	65.5	+1.5
79	116	67.5	-8.2
80	120	66.5	-10.0
81	102	62.5	-18
82	116	67	-5.3
83	111	65	-1.5
84	108	61.5	+7.1
85	101	62	-1.6
86	129	68	-10.0
87	106	63.5	-9.9

APPENDIX—concl'd

	Serial No	Weight (lb)	Height (inches)	Oxygen consumption Per cent
	88	105	63	-15.7
	89	111	64	0.0
	90	119	68.5	-11.2
	91	128	67	-3.2
	92	129	65	-9.4
	93	139	69.5	-6.1
	94	95	61	-16.2
	95	107	64	+4.9
	96	137	70.5	-14.7
	97	84	69	-11.5
	98	89	61.5	+12.6
	99	112	65	-14
	100	110	66	-25
Averages	100	112.5=lb	58.5"	-6.9

A MODIFICATION OF THE ZONDEK-ASCHHEIM TEST FOR PREGNANCY WITH REFERENCE TO THE HORMONE'S EFFECTS ON IMMATURE MALE RATS

BY

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[Received for publication, March 20, 1931]

ALLAN and Doisy (1923) described the vaginal smear method for detection and assay of ovarian hormone. Injection of this hormone into immature or castrated mice or rats is followed in 48 hours by appearance of oestrus. During the oestrus the uterus with its glands hypertrophies and is filled with secretion. These authors showed that the vaginal mucous membrane shares these changes, the vaginal wall becomes thickened and in the basal cell zone of the mucous membrane we find 10-12 layers of polygonal cells the upper part of which has a preponderance of non-nucleated scales. In consequence of this marked change in the mucous membrane, the vaginal smear of the injected animal shows marked differences. A vaginal smear from a castrated rat contains a large number of leucocytes, an occasional elliptical epithelial cell and a varying amount of mucous. If such a castrated rat is injected with ovarian hormone the vaginal smear after 24 hours presents quite a different picture, the leucocytes have completely disappeared and their place is taken by a large number of non-nucleated epithelial scales with nucleated small epithelial cells here and there.

Lowe (1925), Fels (1926) and others demonstrated the presence of the ovarian hormone in the blood and Lowe (1926), Zondek (1928) and others in the urine of pregnant women.

Detection of this hormone in the blood and the urine by the Allan and Doisy test has been utilized by various workers for the diagnosis of pregnancy.

Binz (1924) produced signs of premature puberty in infantile mice following injections of blood serum of pregnant women using 0.5 c.c. for eight days. Siddal (1928) takes 25 c.c. of patient's blood, - separates the serum

which is injected subcutaneously in 1 c.c. doses into an immature virgin white mouse once daily for 4-5 days, on the 6th day a vaginal smear is made. Frank (1928) devised his female sex hormone blood test using 40 c.c. of blood from a vein, the blood is mixed with 30 g. anhydrous soda sulphate and extracted with ether, which is then driven off and the residue dissolved in water. The watery emulsion is then injected in 3 equal parts at 3-4 hours intervals into a castrated adult white mouse, a vaginal spread is made 24 hours after the last injection. Mazel and Hoffman (1929) diagnose pregnancy by injecting 20 mm. of freshly catheterized urine every two hours for five times into castrated mice, a positive vaginal spread is regarded as evidence of pregnancy.

But all these tests are liable to give a high percentage of wrong results as the ovarian hormone level of the blood is raised not only during pregnancy but also in many cases at the beginning of the menopause, in certain cases of amenorrhoea (hyperhormonal form), in some functional disorders and about a week before menstruation. Demonstrable amounts of ovarian hormone appear in the blood and urine from about the eighth week of pregnancy so that besides giving high percentage of erroneous results these tests are not available during the first eight weeks when diagnosis by biological means is most important.

All the workers including Frank, Kingiey and Gustavson (1925) and Zondek and Aschheim (1926 and 1927b) agree that extracts of whole ovary, *corpus luteum* or placenta, which cause a striking and unmistakable stimulation of the tubular genital tract of the animals injected, have no effect on the ovaries of either mature or immature animals. It is therefore clear that there is something outside the ovaries themselves that influences these glands. That intimate relationship exists between the anterior pituitary and the sex glands had long been known through the works of Crowe, Cushing and Homans (1910), Goetsch (1916) and others.

Evans and Long (1922) after administration of anterior hypophysis secretion found the ovaries in injected animals double the weight and containing a larger number of *corpora lutea* than in control litter mates.

The recent work in this direction of Zondek and Aschheim and Philip Smith has conclusively proved that anterior pituitary is the 'motor of the gonads,' and that puberty depends on its secretion activating the gonads.

Zondek and Aschheim (1927a) tried the effects of transplants of every organ of internal and external secretion and injections with unspecific substances on 150 animals but could induce no change in the ovaries. When, however, a small piece (10 mg.) of the anterior lobe of the pituitary (posterior lobe had no effect) was implanted in 3-5 weeks old mice, activity in the ovaries of these animals was produced within the first 100 hours. The changes included hypertrophy of the ovaries, maturation of follicles and presence of *corpora hæmorrhagica* and *corpora lutea* in them. The ovarian hormone coming into action produced the œstrus as shown by a positive vaginal smear and hypertrophy of vagina and uterus.

Phillip Smith (1926) and Smith and Engle (1927) with transplants of pituitary from rats into infantile female rats found that this procedure rapidly induced changes characteristic of sexual maturity (opening of vagina, uterine hyperæmia and distension, follicle and corpora formation). Experiments showed that the anterior pituitary component of the transplant was responsible for this induction of the premature sexual maturity. In the absence of the ovaries, no precocity in development of the uterus or vagina is brought about by pituitary transplants, it is effected by ovarian growth induced by pituitary transplants. Smith (1927b) also found that atrophy of sex organs arising from removal of anterior pituitary could be completely or nearly completely cured by daily pituitary homologous grafts.

This action on the ovaries formed the basis of the Zondek and Aschheim (1928) test for detection and estimation of the anterior pituitary hormone, infantile mice 3-4 weeks old and weighing 6 to 8 are used for the test. Injection of the hormone produces within 100 hours (1) enlargement and ripening of ovarian follicles, (2) mobilization of ovarian hormone and hence the production of the œstius, (3) production of *corpora lutea atretica* (luteinization of granulosa cells in follicles from which the ovum has not been extruded).

Zondek and Aschheim (1928) discovered that during pregnancy, even immediately after conception, there was a striking over-production of the hormone of the anterior pituitary which was found in decidua and placenta and was excreted in urine. This provided the basis of their new test for the diagnosis of pregnancy by the detection of the anterior pituitary hormone in the urine (Aschheim and Zondek, 1928).

Perez (1921), Jung (1922), Gentili (1922) and others have shown that the pituitary hypertrophies during pregnancy. That this increase of function on the part of the hypophysis was essential for the continuation of pregnancy was shown by Aschner (1924) who found that extirpation of the anterior pituitary during pregnancy was followed by abortion. The indispensability of sufficient amount of ovarian hormone for carrying pregnancy to term had already been shown by Andres (1921) who presented a very interesting clinical history to prove that insufficiency of the ovary may be a direct cause of abortion. The patient exhibiting evident symptoms of ovarian insufficiency was a primipara who suffered abortion without apparent cause. Upon becoming pregnant a second time she was submitted to ovarian therapy following which the pregnancy progressed normally until medication was suspended. Four days later a second abortion occurred. A third pregnancy took place and during the whole period of gestation she was treated energetically by the above mentioned treatment. A normal child was born.

The diagnosis of pregnancy by the detection of the anterior pituitary hormone (Zondek-Aschheim reaction) has not the disadvantages common to all tests based on detection of ovarian hormone since the pituitary hormone is present in the blood and the urine from the very beginning of pregnancy.

and is not present (according to Zondek-Aschheim) in any other condition. Fluhmann (1929a) using this test demonstrated the presence of anterior pituitary hormone in the blood of women after bilateral oophorectomy and after radiation castration, and the earlier workers (Tandler and Grosz, Kon, Kolde and Rossle) had demonstrated the pituitary hypertrophy after castration. Engle (1929a) has shown that ovarian response of immature mice and rats following the daily transplants of fresh anterior lobe taken from castrated rats is significantly greater than the response to anterior lobe transplants from normal animals while the pituitary of castrated animals is larger than that of normal. These observations explain the few positive reactions obtained with cases of menopause which so to say is a condition of natural castration, but it is obvious from the predominantly large number of negative reactions with climacteric cases that it is only in a very small percentage of cases that the pituitary hormone level of the blood at this period of life reaches high enough to give a positive Zondek-Aschheim reaction.

While the utility and reliability of the reaction for diagnosing pregnancy has not been questioned, exception has been taken to calling the test an anterior pituitary function test. In other words the claim that it is the anterior pituitary hormone in the urine of pregnant women that stimulates ovarian activity in animals injected with such urine is contested. Engle (1929b) pointed out the differences in ovarian response elicited by treatment with urine from pregnant women and by freshly implanted pituitary lobe. He remarks that either of the two methods of treatment causes follicular growth, but there the comparison ceases. The standard response from fresh gland transplants is follicular growth followed by ovulation, the only luteinization that occurs in the ovary of the immature mouse is subsequent to ovulation. In the response obtained by urine, ovulation has never been secured, instead it is effectively inhibited by atresia of the granulosa and by the formation of the *corpus luteum*. Collip (1930) demonstrated an ovary stimulating hormone of the placenta and he considers it probable that this internal secretion is elaborated in the placenta and is not merely the internal secretion of the anterior lobe of pituitary stored in the placenta. Klein (1930) found hæmorrhagic follicles and *corpora lutea* in the ovary after injection of placental extract, but he did not find the increased thyroid activity described by injections of hypophysin. He thinks that the placenta contains a substance which acts on the ovary but which is not hypophysin. Bacon (1930) found that pregnant hypophysis were poorer in hormone content than the non-pregnant ones, although the hormone content of pregnant blood is vastly greater. He explains this by a possible reduction in activity brought about by a vicarious hormone production in the decidua on analogy to the disappearance of ovarian hormone from the *corpus luteum* of pregnancy with the increase of hormone production by placenta.

Whether the ovary stimulating substance in the urine of pregnant women is anterior pituitary hormone as claimed by Zondek and Aschheim or a placental

hormone as suggested by Collip Kleim and Bacon make no difference in accuracy, reliability and utility of the Zondek-Aschheim reaction for pregnancy. The test has already given conclusive results in hands of various workers. Aschheim (1930) reported 880 cases with 12 failures, an accuracy of 98.6 per cent, these included 410 cases of pregnancy of which 402 gave positive results, 126 cases of amenorrhœa, 12 of chmacteric, 40 healthy women, 60 female patients not pregnant, 10 women with irregular bleeding, 40 cases of disturbances of internal secretions, 20 cases of inflammatory gynæcological conditions, 25 cases of benign ovarian tumour, 35 cases of myomata uteri, all giving negative results, out of 66 cases of carcinoma 64 were negative, the two giving positive results were of genital carcinoma, out of 16 cases from men only 1 gave a positive result and in that case change of urine was suspected, out of urines from 20 cases of internal diseases only one gave a positive result. The earliest cases diagnosed were from 5 to 6 weeks of gestation. He quoted 350 cases tested in the University clinic of Frankfurt with 98 per cent accuracy, 100 cases in Schaafer's clinic with the same percentage, and 50 cases in a women's hospital in New York with one error.

Joffeck (1930) quotes 197 cases of pregnancy in which Zondek got only 4 negative results but, even of these, two examined a second time were positive and the examination could not be repeated with the other two. Allan and Dickens (1930) examined 126 cases definitely known to be pregnant and got 122 positive results, 82 cases known definitely not to be pregnant and got 81 negative results, 6 urines of males all of them reacting negatively, out of 5 cases of menopause only one gave a weak positive result. The earliest case diagnosed was one in which period was overdue 9 days only. Wagner got similar results. Crew (1930) reports 460 cases of pregnancy (for which he had received control information), out of these 446 were returned as correct and 14 as incorrect, of these 14 one was negative according to the physician who sent the urine while the Zondek-Aschheim reaction was positive (repeated three weeks later it gave a negative reaction), in the remaining 13 the Zondek-Aschheim reaction was negative while according to the attending physician it ought to have been positive. The analysis of these 13 cases is rather interesting—4 proved to be cases of dead ovum as in two of them a macerated foetus was delivered 12 hours after the urine was taken and another two were later diagnosed as missed abortion, one miscarried 1 month after and another aborted 15 days after urine was taken, in two cases a positive reaction was obtained on repetition, no detail was supplied in three cases. The earliest cases diagnosed were two within 8 days of the last menstrual period and two within 19 days of it, one within 20 days of the last successful coitus and 5 within 40 days of it.

As shown by Aschner and Andres sufficiency of anterior pituitary, and ovarian hormones in the maternal blood is essential for carrying pregnancy to term, when there is insufficiency of these hormones it is liable to terminate before time and in such cases a negative reaction can well be understood and

perhaps this insufficiency of hormones explains the negative reaction in cases described by Crew who aborted and miscarried

On the other hand the hormone responsible for the test depends for its existence on the vital connection between the placental tissue and the uterine wall, when that ceases on termination of pregnancy it begins to decrease and disappears totally by the 8th day after labour. If that vital connection is lost earlier by death of the foetus (although both the foetus and the placenta may remain in the uterus for the time being) the hormone production will cease and the reaction will become negative and probably this explains of its absence in 4 of Crew's cases who were later proved to be women in whom the foetus had died

Louisa (1928) carried out the test in 132 cases, 87 specimens came from all stages of pregnancy and showed a reaction in 98 per cent of cases. The earliest case that gave a positive reaction was a woman whose menstrual period was 7 days overdue, there were several other women in the first three weeks of gestation all of whom showed a positive reaction. Of 45 specimens obtained from non-pregnant cases only one gave the positive reaction. Erhardt (1929) on the basis of 300 cases confirmed the reliability of Zondek-Aschheim reaction

Briehl (1929) says that a positive result with pregnancy reaction of Aschheim and Zondek gives very definite evidence of pregnancy and a negative result of reaction excludes pregnancy with 100 per cent surety

Brougha and Simmonet (1928 and 1930) extensively tried the reaction for early diagnosis of pregnancy, they found that in all cases when hæmorrhagic follicles and *corpus luteum* followed injection of serum into the mouse the pregnancy existed and in all cases when the reaction was negative the gravid state did not exist

Fluhmann's (1929b) results were rather less encouraging, he examined 100 cases, 48 of pregnancy and 52 controls of the 48 cases of pregnancy he obtained no reaction in 2, reaction for ovarian hormone in 11, and for pituitary hormone in 35, on the other hand in 52 control cases reaction for anterior pituitary hormone was obtained 6 times

Frank (1929) who finds the test disappointing because on one hand he was unable to keep in stock the large amount of immature mice needed for performing the test and secondly, the immature mice regularly died during the course of injections long before a reading could be taken, says that this is by far the best test of pregnancy as yet discovered

The difficulty of keeping a large stock of immature mice is certainly a great drawback, but no worker other than Frank has experienced the high death rate among the injected animals, Zondek and Aschheim had 16 to 17 per cent of their animals dying and in my experiments only 10 per cent of the animals died

I commenced working at this test on the suggestion of my chief, Lieut-Colonel H W Acton, CIE, IMS Director and Professor of Pathology and

Bacteriology, School of Tropical Medicine, Calcutta, in order to test the anterior pituitary function of patients suffering from hyper-keratotic conditions of the skin, e.g., ichthyosis, scleroderma, psoriasis, etc., as Colonel Acton believes that these diseases are due to a hyper-pituitary dysfunction. Only a few of these cases were tested but no changes were found in the ovaries of injected animals after 2.4 c.c. of blood serum injected in doses of 0.4 c.c. each (in cases of pregnancy 0.5 c.c. of the serum gave positive results).

While doing the test according to standard methods (but using rats instead of mice) it was noted that in the tropics maturity amongst the rats is attained rather earlier than in mice in colder climates so that some modification in the minor details of technique are necessary. Before pointing out the differences noted it would be better to describe the test in original

THE TECHNIQUE

Collection of urine—The morning specimen of urine is collected in a clean bottle. Alcohol should not be used for cleaning the bottle or if used should be thoroughly washed away with water as the anterior pituitary hormone is precipitated by this reagent as well as by ether and acetone. The morning specimen before any food or drink is used for the tests as the hormone in the later specimens will be diluted by drinking water, etc. The originators of the test used a voided specimen of urine, but other workers recommended a catheter specimen of urine in preference. If the urine is turbid it should be filtered and its reaction made slightly acid if it is not already so. If the specimen is not to be used for some time, or if it has to be sent by post a drop of cresol to every 30 c.c. of urine is added. It is better to send two specimens, one with cresol and the other without it.

Selection of animals—For each test five or more infantile mice are used as some may die and some may not react. The result is recorded positive even if one of the animals used gives a positive reaction. Aschheim and Zondek take mice from 3 to 4 weeks and weighing 6 to 8 gms. Allan and Dicken used mice of 21 to 24 days age, not above 24 days as Engle and Rosaco (1928) found that mice showed a first oestrus from 28th to 49th day of life.

Injections—Injections are made subcutaneously laterally in the back, care being taken not to enter the peritoneum or pleural cavity, instantaneous death follows such a mishap. An assistant holds the mouse with the left hand by the tail, his right hand grasps one ear with a forceps, the tips of which are protected by a rubber tubing from a blood counting outfit. Labelling of the animals can be done by means of a fine brush and concentrated carbolfuchsin.

The amount of urine to be injected is given in six equal parts, the injections being spread over a period of 48 hours. Zondek and Aschheim give 3 injections on the first day and three on the second. Louria gave 3 injections

a day for 3 days Allan and Dickens distribute their 6 injections over 48 hours as equally as is possible, then times of injections are as follows —

1st day	12 P M	6 P M
2nd day	9 A M	3 P M and 9 P M
3rd day	9 A M	

Doses—Zondek and Aschheim give varying doses to the animals used, the first animal gets 0.2 c.c. for each injection, the second 0.25, the third and fourth 0.3 and the fifth 0.4 c.c. If six animals are used two of them get 0.25 c.c. Allan and Dickens found no special advantage in varying the doses and they give 0.3 c.c. to all the animals. Louisa also gave this dose to all the animals used.

Duration of the test—The animals are autopsied 100 hours after the first injection, i.e., on the morning of the 5th day counting from the first day of injection (if the experiment commenced on Monday animals are sacrificed on Friday morning).

Reading of results—One hundred hours after injection the animals are killed, a vaginal smear made and the abdomen opened. By gently pulling the uterine horn, the ovary appears from under the lower pole of the kidney, together with fatty tissue. The conditions of the ovaries, uterine horns, uterus and vagina are noted. If the red points (to be mentioned hereafter) are not found in the ovary microscopically some workers squeeze the ovary in glycerol and examine macroscopically under a cover-slip. It is better to prepare serial sections of the ovary in all the cases, but some workers recommend this to be done only in doubtful cases, because in the majority of cases the macroscopic appearances are so marked that the result can be safely recorded without utilizing the microscope. For this reason some depend wholly on macroscopic findings, if these are negative the test is repeated.

Interpretation of results—In the untreated mouse of immature age the vagina is a solid cord of cells, the uterus thread-like, the ovaries are pale-greyish pink and hardly the size of a pin-head and microscopically contain only unripe follicles.

Following the injections of urine a positive test is indicated by Macroscopically

- (1) opening of the vagina,
- (2) enlargement, thickening and distension of uterus (Plate XIII),
- (3) ovaries are twice or three times larger and distinctly red and present subiliary yellowish protrusions which correspond to *corpora lutea* or cyanotic protrusions (red dots of varying size) which are due to hæmorrhage into a follicle or *corpus luteum* (Plate XIII)

Microscopically

- (1) vaginal smear shows cornified non-nucleated epithelial scales with varying proportions of nucleated epithelial cells. Section of the vagina shows desquamation (Plate XIII),

- (2) ripe graafian follicles in the ovary (Plate XIV),
 (3) *Corpus hæmorrhagicum* or *corpus luteum* or both in the ovary
 (Plate XIV)

Reactions 1 and 2 (both micro- and macroscopic) are not characteristic of pregnancy as they can be produced by conditions other than pregnancy. Only reaction number 3, i.e., the presence of *corpus luteum* or *corpus hæmorrhagicum* or both is diagnostic of pregnancy. In this connection it is specially to be noted that the presence of ripe graafian follicles only is not to be taken as evidence of pregnancy.*

If only the first two stages are reached in the reaction, the test should be repeated after some days.

In interpreting the test one point is always to be kept in mind, that a positive test is not necessarily a sign of the presence of a fetus but a sign of presence in the body of a highly active source of the hormone responsible for the test such as placenta or its derivatives or some other abnormally active tissue. The test has been proved to show a positive reaction in cases of retained adherent pieces of placenta, incomplete abortion, hydatidiform mole, and chorionic epithelioma. On the other hand the presence of an inactive (dead) placenta is not associated with production of this hormone and so the presence of a dead fetus and placenta does not lead to a positive test. A clinical case of Frank (1929) demonstrates very clearly that the positive reaction can be obtained in a case of retained placenta. Although this test is an ovarian hormone function test, it is worth while to quote this case of Frank's as it brings out the importance of placental tissue.

'Case 1403, A L D was admitted with the history of having been delivered, at a difficult labour, of a dead fetus at full term on 9th May, 1927, after a very disturbed pregnancy during which high blood-pressure and albuminuria had been noted. An irreconcilable difference in the statement of patient and physician existed. The patient asserted that the after-birth had not come away. The doctor on the other hand declared that the placenta had been passed. The patient was admitted 17 days after labour with a uterus reaching one half way to the umbilicus, with little lochia and no foul discharge. A blood test was taken 26th May, 1927, and gave a +3. The complete placenta was manually removed piecemeal. It was found in fresh condition, not specially adherent but evidently in direct contact with the maternal circulation.'

I may now quote my experiences with the test —

While doing the test with infantile rats according to details given by Allan and Dickens (who used mice in their work) the following points of difference were noted

* Aschheim and Zondek have isolated two hormones from the secretion of the anterior lobe, one of which (Prolan A) causes follicle development, the other (Prolan B) causing luteinization of the follicle epithelium. It is the presence of Prolan B in the urine that is diagnostic of pregnancy.

(1) Selection of animals In batches of rats used on 21st day of their age, occasionally control ones (receiving no injections at all) gave a positive vaginal smear and showed hypertrophy of the vagina uterus, and uterine horns. This means that oestrus in these animals (in the tropics) may begin as early as the 25th day after birth. No blood points were observed in these control rats showing hypertrophy of the vagina and uterus, and the test depends only on changes in the ovaries, so that it could be performed with rats of this age, but it is a decided advantage to perform the test with animals at an age when commencing oestral changes are not observed. Keeping this point in view I tried to find a suitable age limit of rats used for the test in the tropics. It was observed that the sex in infantile rats could be differentiated as early as at 10 days of age, but the eyes do not open before they were 16 days old, and unless the eyes were opened the animals could not possibly live independently of their mothers. So the earliest age was 17 days and it was found that rats 17 to 20 days old were the most suitable. These animals at this age weigh from 12 to 15 gs, can stand the injections quite well, and are capable of reacting positively.

(2) Opening of vagina It is stated that in mice used at the 21-24 days age and giving negative results, the vagina was a solid cord of cells while in positive cases it was canalized. No such difference could be observed in rats even with the lower age (17-20 days) as the vagina of all animals whether giving positive or negative results or kept as controls was found canalized. However, there was this difference that the external vaginal opening was not patent in the control and negative animals (used at 17-20 days) while it was patent in positive cases.

(3) Thickness of uterus In case of rats (even with the lower age 17-20 days) although the uterus in the control and negative cases was decidedly thinner and less developed than in positive cases it was never as thin as a thread.

(4) The time of injections used by Allan and Dickens was rather inconvenient as it requires injection to be given at 9 P.M. on the second day. The first few experiments were done according to these authors' times but later the six injections were distributed over three days giving two injections a day, one in the morning and the other in the evening, as it was found that results got with this procedure differed in no way from those got with Allan and Dickens' timing. Three injections a day for three days were tried after Louisa but no definite improvement in results was noted.

The following points were in addition noted in connection with the test —

(1) To find whether the pituitary hormone contained in urine and serum of pregnant women and affecting the precocious sexual maturity in infantile rats contained any growth producing factor both the controls and animals actually used in the test were accurately weighed at the beginning and end of the experiment and it was observed that there was no difference in the average increase in weight in uninjected and negative cases on one hand and

positive cases on the other. From this it can be concluded that no growth factor is present in the hormone in the serum and urine of pregnant women. This finding is in concurrence with the results arrived at by Evans and Simpson (1928) that the urine of pregnant women contains the maturity provoking and not the growth promoting hormone of the anterior pituitary. Laquer (1929) also did not find the growth provoking factor in the hormone present in urines of pregnant women.

(2) Taking in view the comparative ease in this country of getting a sample of blood compared with a catheter specimen of urine, the test was performed with blood serum simultaneously with urine, and it was noted that in most cases results with serum were more marked than with urine, apparently due to the hormone being in a greater concentration in the blood than in the urine. Five injections of 0.1 c.c. of serum gave positive results, doubling the amount, i.e., giving 0.2 c.c. for each injection did not make any appreciable difference in the reaction.

Serum gives more marked results, is easier to obtain than a catheter specimen of urine, can be obtained at any hour the case is seen (in case of the urine only the morning specimen can be used), serum is convenient for sending by post and is less liable to decomposition and its consequent toxic effects on injected animals. With serum, moreover, it is possible to reduce the number of animals used. Originally five or more animals were used for each test, because some may die and some may not react. Realizing the difficulty of breeding a large number of infantile rats and encouraged by the low percentage of deaths amongst our animals combined with the fact that no animal injected with blood serum died and that serum gave more marked results, in the later experiments I used only two rats for each test and a positive result always occurred whenever the woman was pregnant. If two animals were to be injected with serum it would require only 1 c.c. of it for both or one could be injected with serum (only 0.5 c.c. required) and the other with urine (not necessarily a catheter specimen). In this connection it is to be pointed out that voided specimens gave a slightly higher death rate than the catheter specimen. In the animals injected with urine after catheterization deaths occurred in two experiments, whilst in one of these the whole five animals died suggesting that there may have been some toxic substance present in the urine. In the urine injected after being passed the deaths among the animals were scattered in the series. Tricresol or any other antiseptic was never used either for catheter or voided specimens, urines when not in use were kept in the ice chest or cool room.

(3) Efforts were made to reduce the duration of the test and it was found that with larger doses injected at shorter intervals the reaction could be got at least on the morning of the 4th day, but the standard method is certainly better. As already observed Crew (1930) has evolved an emergency test whereby he can record positive results within 60 hours. Ehrhardt (1929b) has tried various procedures in the mouse to shorten the time necessary for carrying

out the test These included splenectomy, unilateral ovariectomy, partial or complete removal of the uterus and keeping test mice in warm surrounding (32° – 40°C) None of these were successful Implantation of other ductless glands along with the anterior lobe of the pituitary gave inconstant results as also did injections of anterior pituitary hormone, along with adrenalin, hypophysin, insulin, and thyroxin

(4) Labelling the rats by means of a fine brush and India ink was tried, the marks nearly disappear on the 2nd or 3rd day, it may be that the animals in the same cage lick this off from each others' body They have to be marked at least two or three times The method of snipping off small segments from different portions from the ears of rats as suggested and used by Frank (1929) was found to be very satisfactory

The vaginal smear is made with the help of a platinum loop, it is passed into the vagina deep enough to reach its upper limit, then withdrawn and the loop rubbed on a clean slide

I have already referred to the difficulty of breeding large number of infantile rats specially because I have experienced the tendency of several mother rats to destroy their litters while very young, sometimes this experience is very annoying as several successive batches are destroyed like this And then out of the survivors the male ones had to be rejected so that the supply was further cut down There is no place here from where rats of known age could be obtained The very limited supply of infantile female rats of proper age coupled with the fact that the age limits between which these animals can be used for the test are rather narrow, makes it almost impossible to perform the test unless carried out on a large scale as very large number of rats would have to be kept to insure a regular supply of animals of the proper age on any day Another point is that the test cannot be available to the physicians who only require its help every now and then as it would not pay to keep up this breeding in order to do a few tests from time to time In my attempt to solve these difficulties I turned to male rats and tried the effects of the urine and serum from pregnant women on them The results were distinctly encouraging

It was noted that in infantile male rats after injections with urine from pregnant women (three injections of 0.3–0.4 c.c. thrice a day for two days) there was definite increase in the size of the testicles, seminal vesicles, prostate and Cowper's glands and penis as compared with controls and above all the testicles in the injected animals descended into the scrotum while in the controls they were still in the abdomen (Plate XV) The descent of the testicles can be noted as early as on the fourth day after beginning the injection and becomes better marked on the 5th This reaction can be obtained in rats from the 21st day to such time as the testicles descend (In two rats watched up to 49 days of age the testicles descended in one at six weeks of age while they had not descended in the other It requires further observations on a large number of animals to be able to say definitely the age at which the testicles

usually descend) Microscopically the seminiferous tubules in the testes are wider, and the spermatogenous epithelium is more active (further observations are needed to find when mature spermatozoa first appear and by how many days the injections can anti-date this maturity) There is marked hyperplasia in the prostate, seminal vesicles, and Cowper's gland

The effect of injections on older rats with descended testicles was tried, in these the injections increased the size of the testicles, seminal vesicles, Cowper's and prostate glands (Plate XVI)

On adult male rats with fully developed and descended testicles the effects of injections were variable, in one case there was no appreciable effect, in the other the scrotal prominence became much more marked and hyperæmic and in a third there was definite regressive effect, the testicles were smaller and had become abdominal but the penis was hyperæmic as compared with the control

Turning to the literature on the effects of the pituitary anterior lobe transplants or feeding and injections of urine from pregnant women one finds that Phillip Smith (1927*b*) observed that daily pituitary homologous grafts hasten the growth of the genital system in the immature male rat There is no marked effect on size of the testicle in the younger animal The genital system exclusive of testicles, however, shows a marked increase With increase in age and more prolonged treatment the testicular response is also marked Smith and Engle (1927) found that the genital system of the male mouse responds to pituitary homo- and hetero- transplants, the response of the testis is not so marked and is variable while the remaining organs increase in weight and activity They showed that implanted pituitary affected the genital tract through inter-mediacy of the testes, for in their absence no growth response resulted in the remainder of the genital tract Goetsch (1916) by anterior pituitary feeding in male rats observed marked increase in weight and active development of genitalia and an entire complete descent of testicles which even on gross inspection and palpation were definitely larger than those of control male Zondek and Aschheim (1927*b*) found that injection of the anterior lobe pituitary hormone derived from urine of pregnant women produced only a slight enlargement of the testes, but a 3 to 5 fold increase in the size of the vesiculæ seminales Engle (1929*c*) working with immature male rats observed marked hypertrophy of sex apparatus after injections of urine from pregnant women Bougha and Simmonet (1929) report that the urine of women at the beginning of pregnancy stimulates the development of the genital tract in the male mouse before puberty and causes hypertrophy of accessory glands in adult males They say that after 3 months' pregnancy folliculin becomes prominent in the urine and overshadows the hypophysical secretion so that such urine has an inhibitory influence on the genital tract Fels (quoted by Korenchevsky, 1930) on the other hand injected infantile male mice with serum from pregnant women, the testes were smaller than normal

though histologically the amount of interstitial cells was increased, the prostate and seminal vesicles were hypertrophied

The literature on effects of extracts of anterior pituitary on the male genital organs is rather conflicting, some workers reporting a stimulating and others a regressive action. This conflicting nature of findings may, however, be reconciled when one takes into account the observations of Evans and Simpson (1928). Their work established the presence of two hormones in the anterior pituitary secretion, one growth promoting and the other maturity provoking, alkaline extracts of anterior pituitary being effective in stimulating growth and acid extracts in provoking maturity. They further observed that the effects of the maturity provoking hormone can be completely nullified by simultaneous administration of the growth provoking hormone. Putnam (1929) and Hewitt (1929) also showed the presence of these two separate hormones in the anterior pituitary. Results in cases where an alkaline extract is used would therefore naturally differ from those in which acid extract is used. To bring out the fact that results will differ according to technique, more clearly it would be better to quote a concrete example. Evans and his co-workers in 1926 found that an extract of hypophysis delays sexual maturity in the male rat, autopsy of two treated male rats, when compared with their controls showed great diminution in testes weight. But after establishing the presence of two separate hormones in the anterior pituitary they observed that in the hypophysis there was not a gonad depressing but a gonad stimulating hormone, they explained their previous contradictory results by the fact that the growth promoting hormone completely nullifies the effect of gonad stimulating hormone when simultaneously administered.

As already noted, Bougha and Simmonet (1929) report that after 3 months' pregnancy folliculin becomes prominent in the urine and overshadows hypophysical secretion so that such urine has an inhibitory effect on the male genital tract. My experience on this point is quite different, with 16 specimens of urine from pregnancies of 4th, 5th, 6th, 7th and 9th months never was any regressive effect observed on immature rats. Effects of three of these specimens of urine were tried on adult male rats with variable results, in one case there was no appreciable change, in another there was a stimulating influence, while in the third a regressive effect was noted, the testicles became smaller and travelled back into the abdomen. No relation between the period of pregnancy and extent of stimulation was noted. In one case the urine from a 7-month case of pregnancy gave a less marked result than that from a 2 months' pregnancy (both tests were done side by side) but in other cases urines from the 7th month gave quite marked results and one case 36 weeks pregnant gave better results than another of 2 months only (both tests were done side by side). It requires observations on an extensive scale to be able to reach any conclusions on this point. For the present it will be remembered that two hormones are excreted in the urine of pregnant women, ovarian and anterior

pituitary, the ovarian hormone slowly increases in the first eight weeks after which time it grows quickly giving the largest amount in the last two months, while on the 8th day after delivery urine is quite free from it. The pituitary hormone is present in large quantity on 5-7 days of conception, and increases in amount until 8th month, after which it is reduced and disappears entirely on the 8th day after delivery. So that during the first 2 months of pregnancy the only hormone in the urine to have its effect on the injected animals is the anterior pituitary. After this the ovarian hormone also comes into play to exert its action on the male genital system. That ovarian hormone exerts an anti-masculine effect was first shown by Goetsch and Hermann and Stein. Goetsch (1916) with *corpus luteum* feeding observed that the testes became smaller in size and weight. Frank (1929) quotes the work of Hermann and Stein who found that hypoid extract of *corpus luteum* not only exerted an inhibitory effect on the growth of immature testes of rabbits but also on spermatogenesis. In older animals if spermatogenesis had begun regression took place. Truffi (1927) and Laquer (1927) have observed a definite anti-masculine effect exerted by ovarian hormone. Golding and Ramirez (1928) found that in male rats getting injections of ovarian hormone the size and weight of the testicles was one-half or one-third of its control litter mate. More noticeable than this, however, was the fact that in animals receiving injections, the testicles remained in the abdomen while in controls they descended into the scrotum.

On the other hand Fellner (1921) found that injections of placental and ovarian lipoids as also of testicular lipoids produced a diminution of testes. He concluded that the action was not specific since other lipoids produced it. Frank (1929) while referring to the anti-masculine action of ovarian hormone remarks that such apparently conclusive evidence must be accepted with caution and reserve. In this connection he refers to the works of Heredia and Kauders. Heredia found that male chickens fed with bull testes had testes smaller than the controls, Kauders fed testicle extracts in overdosage and obtained atrophy of the rat testes. Frank concludes that these results of Heredia and Kauders speak for sensitiveness of the male gonad to many influences rather than to any specific contra-sexual effect.

Injections with the urine from males have a slight stimulating effect on the genital tract of male immature rats. This stimulating effect with male urines was also observed by Engle (1929c).

Change in thymus—The thymus has been called the 'gland of infancy' and it regresses with onset of maturity *pari passu* with the growth of the genital organs. Any procedure stimulating the genital organs would therefore be expected to cause a decrease in size of the thymus. Working on this idea this gland was dissected in all the injected and control animals that were sacrificed. It was found that both in males and females the thymus in the injected animals was always smaller (at least by one-third and sometimes to a more marked extent) than in the controls. This atrophy of the thymus in injected animals

was more marked in younger than in older animals. Golding and Ramirez (1928) working with ovarian hormone found the thymus of the injected animals (both male and female) invariably smaller than that of the control. Hering (1920) found that during pregnancy the thymus had undergone rapid involution and was much diminished in size.

The modified test—The changes in the male genital tract produced by injections with urine from pregnant women make the basis of the modification that is proposed in this paper in the Zondek-Aschheim test for pregnancy.

TECHNIQUE

1 A specimen of blood serum or a morning specimen of urine is obtained. If possible, it is better to get a catheter specimen and to keep the urine in an ice chest or cool room.

2 Young male rats of about equal size from 15 to 50 grammes in weight and with undescended testicles are procured from the market. Three animals in each case will suffice.

3 One of the animals is kept as control and the other two are injected thrice daily for two days with serum or urine (0.4 c.c. of urine or 0.2 c.c. of serum for each dose), i.e., six injections in all.

4 The result is looked for from the 4th day after beginning of the test (in one case I could note it on the 2nd day and in another on the third day), it is better marked on the 5th. The animal is held on its back and the prominence (caused by descent of the testicles) and hyperæmia of the scrotum noted. A positive result in contrast with the control is very marked, the descended testicles (which can be palpated by hand) and hyperæmia of the scrotum contrasting with the barely noticeable prominence in the region of the scrotum. The penis is also developed to a greater extent in the positively reacting animal than in the control.

It will be observed that male rats weighing from 15–50 gs. can be used for the test, this wide range of limits makes it possible for the test to be performed on young rats (all of them of about the same weight and size) bought from the market. This does away with the necessity of breeding animals in the laboratory and makes the test available to those who have to perform it only now and then. The test is further simplified by the fact that it is not necessary to sacrifice and dissect the animals used, the prominent bulging caused by the descent of testicles and the hyperæmia as compared with practically no bulging in the control is sufficient evidence of the test being positive.

The number of cases examined is rather small, only 50 tests have been performed. In 21 cases female rats were used, in 28 male rats and in 7 both male and female. Of the 50 cases 39 were known to be pregnant, 3 females with inflammatory gynaecological conditions, 4 men with skin diseases and 4 healthy males. Injections with serum from skin cases had no effect on the ovaries of the female rats. Injections with urines from gynaecological cases had no effect on male rats, urines from healthy males had a stimulating effect.

on the genital system of male rats. Of the 39 cases of pregnancy positive results were obtained in 36 cases (amongst these was included a case of ectopic pregnancy) and 3 gave negative results. Of the three cases giving negative results two were five months pregnant and in the third only one period was missed. In one of the cases in the 5th month, the doctor, on inquiry, reported that foetal heart sounds were not heard, that the uterus was not increasing and that the lady was getting a red vaginal discharge, a few days afterwards a dead foetus was delivered. The other case in the 5th month miscarried a few days after urine was taken. The lady who had missed one period only when blood was taken missed another period but within a few days of missing this second period, she lost some blood *per vaginam* and it was thought that she had begun menstruating and was not a case of pregnancy at all. But three or four days later the placenta was delivered showing the case to be one of abortion.

The 28 cases tested with male rats includes 21 known to be pregnant, 4 healthy men and 3 women with inflammations of the tubes. Of the 21 cases of pregnancy two gave negative results, one of these aborted and the other miscarried a short time after the test was performed (these cases have been referred to above) and 19 gave positive results. The urine from the cases of inflammation of the tubes gave negative results, the urines from men had some stimulating effect on the genital tract of the male immature rats but it was not so marked as in case of pregnancy.

Before finishing I wish to express my gratitude to my chief Lieut-Colonel H. W. Acton, C.I.E., I.M.S., Director, School of Tropical Medicine and Hygiene, Calcutta, whose pupil I have the privilege and honour to be. This work was commenced at his suggestion and throughout he has been a source of encouragement and inspiration to me. My thanks are due to him for allowing me to publish my findings.

SUMMARY

1 Due to earlier maturity of rats in the tropics it is suggested that female rats of lower age (17-20 days) be used for the Zondek-Aschheim reaction. It is observed that the vagina is canalized and is not a solid cord of cells in animals at this age.

2 The test was carried out with the serum of pregnant women with satisfactory results.

3 The effects of injections of urine from pregnant cases on the genital system of male rats are described. It is shown that in immature animals the testicles hypertrophy and descend into the scrotum and that the seminal vesicles, prostate, and Cowper's glands and penis hypertrophy. In older rats with descended testicles the hypertrophy of the testicles, seminal vesicles, Cowper's and prostate glands and penis is marked. Effects on adult rats are variable.

4 Based on these findings a modification of the Zondek-Aschheim test using male rats is proposed. Descent of the testicles in injected animals is looked for, this makes it unnecessary to sacrifice the animals. The modification does away with the necessity of getting animals of definitely known age and hence the need of breeding animals in the laboratory.*

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* While the paper is in press Reinhart and Scott (1931) have published a modification of the Zondek-Aschheim test in which they remove the breeding difficulty by using female rabbits for the test. Advantage is taken of the fact that the female does not ovulate without copulation. A female rabbit over three months old and not pregnant is required. Five c.c. of the urine to be tested is injected into the marginal ear vein of the rabbit and a laparotomy is performed after 24 hours and the ovaries examined for *corpora hæmorrhagica*. The presence of *corpora hæmorrhagica* constitutes a positive reaction.

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PLATE XIII

Positive

Normal



Fig 1



Fig 2

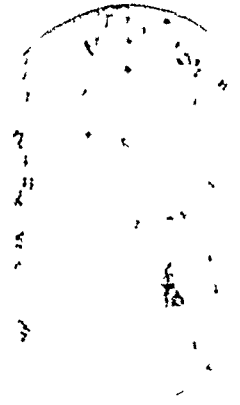


Fig 4

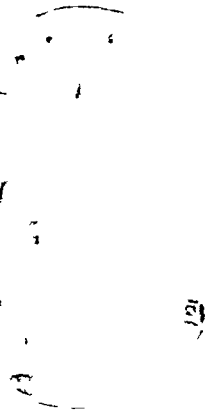


Fig 3



Fig 5



Fig 8



Fig 10

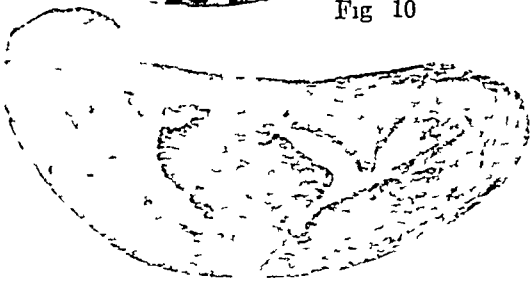


Fig 12



Fig 6



Fig 7



Fig 9

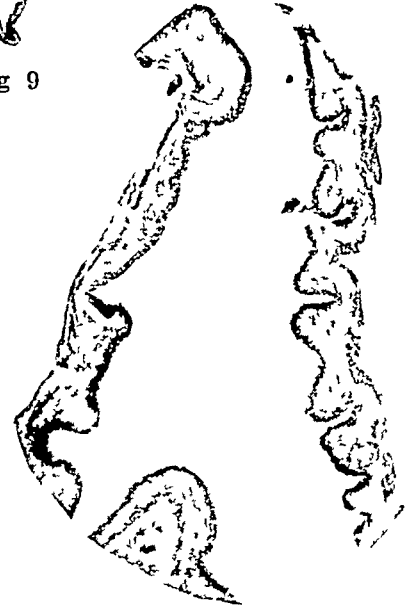


Fig 11

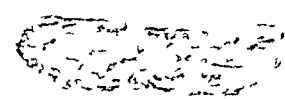


Fig 13

EXPLANATION OF PLATE XIII

- Fig 1 Female rat injected at age of 18 and autopsied at 22 days. Note the presence of the external vaginal opening (Natural size)
- „ 2 Litter sister, received no injection. Note the absence of external vaginal opening (Natural size)
- „ 3 Vaginal smear from the injected rat shown in Fig 1. Note the non-nucleated epithelial scales and nucleated epithelial cells (positive vaginal smear) (Objective 1/6th in, ocular $\times 2$)
- „ 4 Vaginal smear from a rat of 26 days, injected at the age of 22 and giving a negative reaction. Note presence of mucous pellets (Objective 1/3 in, ocular $\times 2$)
- Figs 5 and 6 Thymuses, ovaries and tubular genital tracts of two rats of 25 days age from the same litter (Natural size)
- Fig 5 Injected with urine from a case of pregnancy. Note the comparative atrophy of the thymus. Hypertrophy of the ovaries and blood points on their surface
- Fig 6 Received no injections. Note large size of thymus, small size of ovaries and absence of blood points on their surface. Uterine horns, uterus and vagina are developed to a somewhat greater extent (the animal was in oestrus). This clearly shows that oestral changes may commence as early as the 25th day after birth
- Fig 7 Thymus, ovary and tubular genital tract of an untreated rat of 22 days (Natural size). Note the large size of the thymus and that the vagina is canalized
- „ 8 Vagina of treated rat (shown in Fig 1). Note hypertrophy of the vagina, thickness of the mucous membrane and free scales in the lumen (Objective 1 in, ocular $\times 2$)
- „ 9 Vagina of untreated litter sister (Objective 1 in, ocular $\times 2$)
- „ 10 A portion of (8) highly magnified to show layers of polygonal cells topped by scaly layers and free scales in the lumen (Objective 2/3 in, ocular $\times 4$)
- „ 11 A portion of (9) highly magnified. Note absence of polygonal cells and scales (Objective 2/3 in, ocular $\times 4$)
- „ 12 Uterus of treated rat (Objective 1 in, ocular $\times 4$)
- „ 13 Uterus of untreated rat (Objective 1 in, ocular $\times 4$)

EXPLANATION OF PLATE XIV

- Fig 1 Ovary of a treated rat showing fully formed *corpus luteum*
(Positive reaction) (Objective 2/3 in, ocular $\times 2$)
- Figs 2 and 3 Ovaries of treated rats showing *corpus luteum* in formation
with remains of the hæmorrhage in the centric (Positive reaction)
(Objective 2/3 in, ocular $\times 2$)
- Fig 4 Ovary of a treated rat showing ripe graffian follicles (Negative
reaction) The case, urine from which gave this reaction, aborted
shortly afterwards (Objective 2/3 in, ocular $\times 2$)
- „ 5 Ovary of an untreated immature rat of the same age showing unripe
follicles (Objective 2/3 in, ocular $\times 2$)

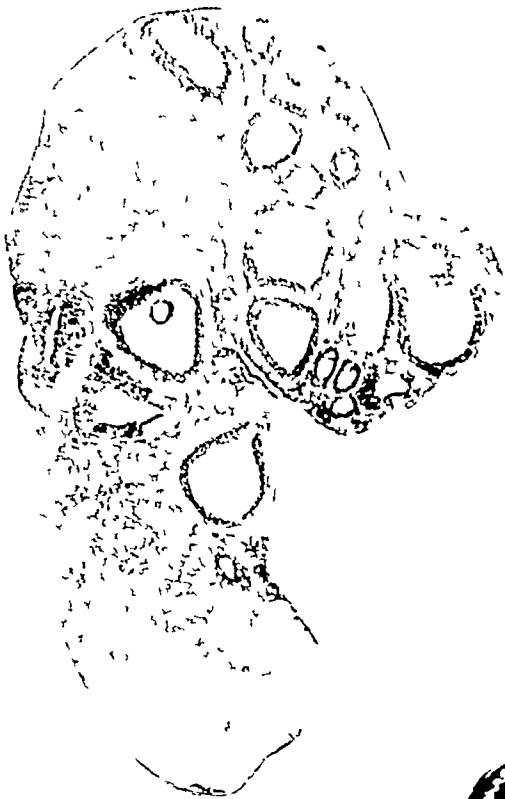


Fig 1

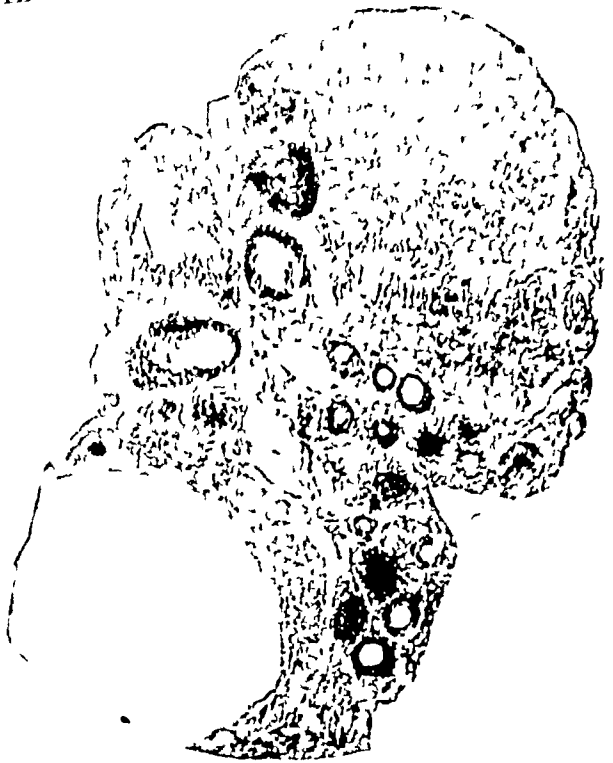


Fig 2



Fig 4

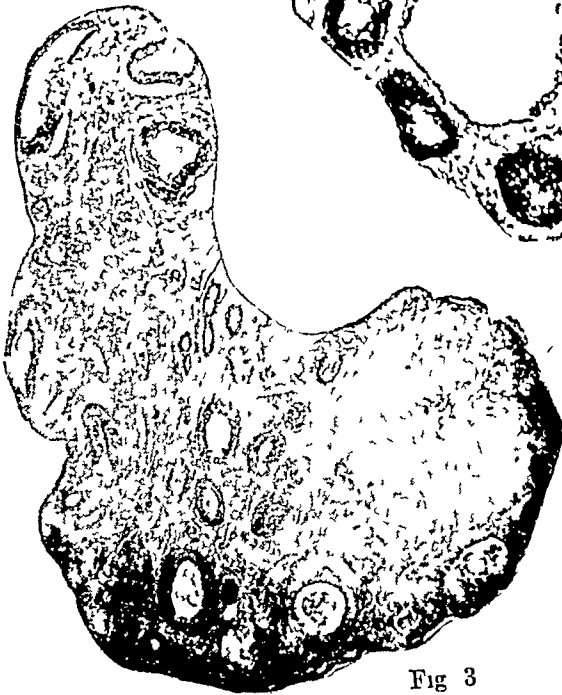


Fig 3



Fig 5

EXPLANATION OF PLATE XV

- Fig 1 Male rat injected with urine at age of 28 days Note the descent of the testicles and bigger size of penis (Natural size)
- „ 2 Uninjected litter brother (Natural size)
- „ 3 Sex organs and thymus of injected rat (shown in Fig 1) Note size and descended position of testicles Bigger size of penis, Cowper's and prostate glands and smaller size of thymus (Natural size)
- „ 4 Sex organs and thymus of uninjected rat (shown in Fig 2) Note the size and abdominal position of testicles The empty scrotal sac on one side of the penis (that on the other is not clearly seen) Smaller size of penis, Cowper's and prostate glands and bigger size of thymus (Natural size)
- „ 5 Three turned over to show marked increase in size of seminal vesicles
- „ 6 Four turned over to show the smaller size of seminal vesicles

Normal



Fig 1



Fig 2

Positive



Fig 3

Seminal
Vesicles



Fig 5

Urinary
bladder
Prostate

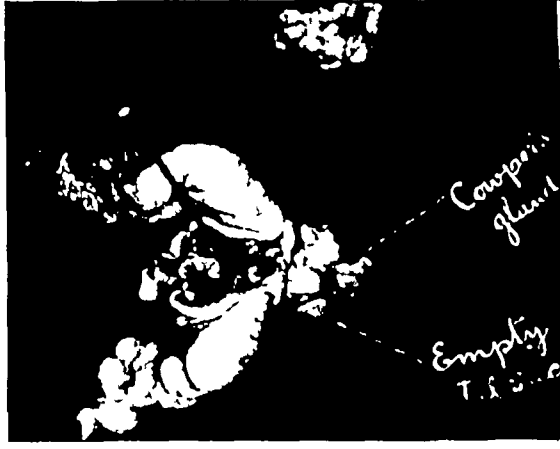


Fig 4

Seminal
Vesicles



Fig 6

EXPLANATION OF PLATE XVI

- Figs 1 and 2 Two sub-adult rats of about equal size and testicular growth
- Fig 1 Received injections of urine from a case of pregnancy Note the greater prominence of the testicles and the larger size of penis (Natural size)
- „ 2 Received no injections (Natural size)
- Figs 3 to 6 Sex organs and thymus from above rats (Natural size)
- Fig 3 Note larger size of testicles and penis Cowper's and prostate and seminal vesicles and smaller size of thymus
- „ 4 Note smaller size of testicles, penis, Cowper's and prostate glands and seminal vesicles and larger size of thymus
- „ 5 Organs shown in 3 turned over to show marked increase in size of seminal vesicles
- „ 6 Organs shown in 4 turned over to show size of seminal vesicles for comparison

PLATE XVI

Normal

Prostate



Cowper's gland
Fig 1

Seminal
vesicles



Fig 6



Fig 2



Fig 1

Positive

Prostate



Cowper's gland
Fig 3

Seminal
vesicles



Fig 5

EXPLANATION OF PLATE XVII

- Fig 1 Section of testes from a rat injected at age of 28 days Note the wider seminiferous tubules and active spermatogenous epithelium (Objective 1/6 in, ocular $\times 2$)
- „ 2 Same from a litter brother who received no injections (Objective 1/6 in, ocular $\times 2$)
- „ 3 Section of prostate from a young injected rat (Objective 1/6 in, ocular $\times 2$)
- „ 4 Same from another young rat of about equal size who received no injection (Objective 1/6 in, ocular $\times 2$)
- „ 5 Section of Cowper's gland from an injected young rat (Objective 1/3 in, ocular $\times 2$)
- „ 6 Same from an uninjected one of about the same size (Objective 1/3 in, ocular $\times 2$)
- „ 7 Section of seminal vesicles from a rat injected at age of 49 days (Objective 1 in, ocular $\times 2$) Note the general hypertrophy and hyperplasia of the lining epithelium
- „ 8 Same from a rat of about equal size who received no injections (Objective 1 in, ocular $\times 2$)

Positive

Normal



Fig 1

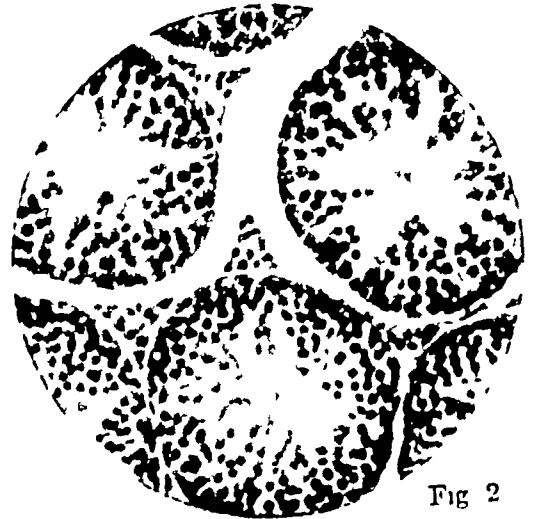


Fig 2

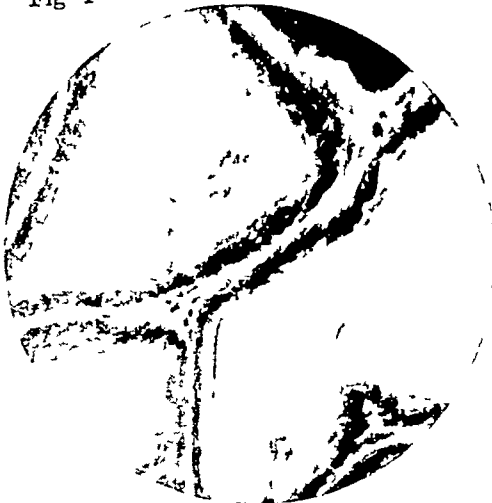


Fig 3

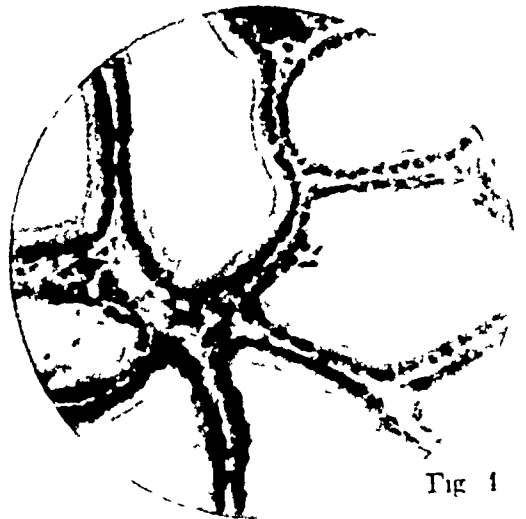


Fig 4

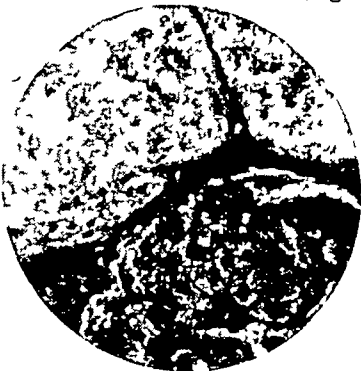


Fig 5



Fig 6



Fig 7

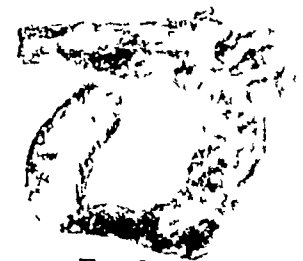


Fig 8.

STUDIES IN GASTRIC SECRETION

Part I.

BY

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[Received for publication, March 30, 1931]

VARIOUS methods have been employed from time to time for the study of gastric secretion in man and animals. Earlier observers were met with the difficulty of obtaining a pure sample of gastric juice. Perforated metal or wooden capsules, to which strings were attached, were used. These were filled with food, introduced into the stomach, and afterwards withdrawn. In some cases, the capsules were made to be vomited out, or passed *per rectum*. Sponges with strings attached were also used for this purpose. Although the method was crude, it gave useful information in the hands of Reaumur and Spallanzani, who showed that the gastric juice is acid, that it prevents putrefaction, and that it digests food *in vitro*.

Later observers (Piout, Tiedemann and Gmelin) killed their animals, either after introducing some indigestible material in the stomach (such as pebbles), or in the course of digestion of a meal. By such a method they demonstrated the presence of hydrochloric acid in the gastric juice, and showed that the acidity was really due to this.

Then followed the classical observations of Beaumont, who collected the juice from a case of gastric fistula. These investigations were made on Alexis St Martin in 1822 who, as the result of a gunshot wound, developed an opening between the stomach and the exterior. Beaumont's researches gave a great impetus to the study of the functions of the stomach in health and

disease. It was his work also which led to the introduction of gastrotomy as a surgical procedure in experimental physiology. Heidenhain separated a small pouch from the main body of the stomach, and was the first to obtain pure gastric juice in this way. This procedure was modified and perfected by his pupil Pavlov, who obtained an isolated stomach pouch with its nervous connections intact. Thus a sample of the secretion was obtained from this pouch which was comparable with the normal secretion in every way. Pavlov analysed the juice and studied the influence of various dietetic, nervous, and other factors on its secretion.

The method, however, which *par excellence* has proved most applicable to man is the one in which a tube is introduced into the stomach. According to Garrison, the discovery and application of the stomach tube and stomach pump go back into antiquity. In modern medicine it originally appears to have been used for artificial feeding and gastric lavage. Leube and Kulz were the first to use the tube for obtaining a sample of gastric juice for purposes of analysis. They employed the principle of fractional analysis, removing a small portion of the test meal at definite intervals. They were thus able to study the variations in acidity and in the amount of pepsin at different stages of digestion.

The chief obstacle to the clinical application of this method on a wide scale was the discomfort caused by swallowing a tube which had a large calibre, and was not flexible enough. It could not be retained easily in the stomach for any length of time. Soft flexible catheters were therefore used from time to time for this purpose. But the practice remained in the background till the modern method of fractional analysis was introduced by Ehlmann, Ehlennich and Ettinger in Germany, and Rehfuss and others in America.

MODERN METHODS OF GASTRIC ANALYSIS

The more important methods of analysis of gastric secretion in vogue at the present day are —

- 1 Ewald's method
 - 2 Rehfuss's fractional method
- 1 *Ewald's method is as follows —*

After a night's fast, the patient is given in the morning a test meal, which consists of a pint of tea and a small piece of toast ($\frac{1}{2}$ oz). One hour after the test meal, a stomach tube is introduced, and as much as possible of the stomach contents withdrawn with a syringe. The fluid removed is submitted to both qualitative and quantitative examination.

(a) Qualitative analysis

The volume, smell, colour and the presence of blood, bile and mucus are noted. The fluid is filtered and the following tests are performed —

- (1) Test for free hydrochloric acid by Gunzberg's reagent

- (ii) Test for lactic acid by Uffelmann's reagent
- (iii) Test for blood This is carried out with the precipitate An ethereal extract of the solid scrapings from the filter paper is prepared Production of blue colour by the addition of alcoholic guaiacum solution and hydrogen peroxide indicates the presence of blood

(b) *Quantitative analysis*

The following estimations are made —

N/10 alkali (NaOH) with Töpfer's reagent as indicator

- (i) Estimation of total acidity by titrating the above still further with N/10 alkali (NaOH) with the addition of phenolphthalein as indicator
- (ii) Estimation of total chlorides The neutralized juice of experiment (i) above is evaporated to dryness, and the residue incinerated The amount of total chlorine in it is estimated by Volhard's method
- (iii) Estimation of mineral chlorides Without previous titration, a measured quantity of gastric juice is evaporated to dryness The residue is incinerated In the mixture of inorganic salts thus obtained, the chlorides are estimated by Volhard's method

In this way, all the hydrochloric acid is volatilized off, and the result represents mineral chlorides

- (iv) Estimation of active hydrochloric acid This is obtained by subtracting mineral chlorides from total chlorides
- (v) Estimation of protein hydrochloric acid This is obtained by subtracting free hydrochloric acid from active hydrochloric acid

2 *The fractional method of Rehfuess* is as follows —

A light supper, consisting of a glass of milk and a charcoal biscuit, is taken at night The gastric analysis is carried out the following morning before taking any food or drink A stomach tube is swallowed, and the gastric contents completely removed by means of a record syringe This is the specimen of the *resting gastric juice* It is set aside for examination

While the tube is still retained in the stomach, the patient is made to swallow the test meal, which consists of a tablespoonful of oatmeal boiled in a quart of water, until the quantity is reduced to one pint It is strained through a piece of muslin

After swallowing a pint of this test meal, specimens are withdrawn by means of the record syringe afterwards at intervals of 15 minutes for two to two and a half hours afterwards or until the stomach is empty If at the end of two and a half hours the stomach is not empty, all the contents are

completely removed at that time. The quantity removed at each 15 minutes interval is 15 c.c. The examination is carried out as follows —

(a) *Examination of the resting juice*—The volume, colour, and smell are noted. The presence of charcoal, blood and mucus, bile and lactic acid is looked for. The specimen is then filtered and the following estimations are carried out —

(i) *Free hydrochloric acid*—This is estimated by titrating the filtrate with N/10 alkali (NaOH) with Toepfer's reagent as indicator.

(ii) *Total acidity*—After the above titration, phenolphthalein is added to the same fluid as an indicator, and the titration is continued with N/10 alkali (NaOH) till pink colour is obtained.

The chlorides are not estimated, as in Ewald's method.

(b) *Fractional analysis*—The samples of gastric contents removed at intervals of 15 minutes are examined for free hydrochloric acid and total acidity in the same way as described above. At the same time, tests are carried out for the presence of starch by means of a dilute solution of iodine. Appearance of blood, bile and mucus is also noted. Curves are charted out for the variation in the amount of free hydrochloric acid and total acidity during the period of observation.

Here again, the chlorides are not estimated as in Ewald's method.

Both the methods yield useful information. The chief advantages of the fractional method of Rehfuess are —

(1) The resting juice is examined, a knowledge of which by itself is of great physiological and pathological interest.

(2) The fractional analysis gives a valuable graphic picture of the variations in the free hydrochloric acid, and total acidity at different stages of digestion. This is not available in the one hour method of Ewald.

In our investigations we employed the fractional method of Rehfuess throughout.

It is of interest to consider briefly the different types of stomach tubes in vogue at the present day.

STOMACH TUBES

Einhorn's duodenal tube—This is a long rubber tube with a metal piece at one end. The metal tip is made of hard steel, olive in shape, plated with silver, weighing 48 grains, and having small round perforations. The rubber tube has a diameter of the size of No. 8 catheter. It is marked with circles at distances of seventeen inches, twenty-four inches, and thirty and a half inches from the tip, indicating the distance from the teeth to the cardiac end, pyloric end and the duodenum respectively.

The perforations in the Einhorn's tube are very minute. They readily become occluded with mucus and alimentary debris, resulting in small foamy specimens insufficient for analytical purposes.

Palefski's modification—Palefski, recognizing the above difficulties in taking out the gastric juice, modified the tip in the Einhorn's tube, so that instead of weighing 48 grains, it weighed 108 grains. It was gold plated. This could be swallowed more easily, and it passed more readily into the duodenum.

The disadvantage in both, however, is that the metal tip may separate out from the rubber tube, and this, if it happens in the stomach, is a very undesirable thing.

Rehfsuss's tube—This is the same as the Einhorn's tube with a slight modification in the metal tip. It is made of hard steel or manganese bronze, and is bulbous in shape, so as to be easily swallowed. It weighs 90 to 120 grains and is slotted instead of perforated. The slots are so cut, that their diameter is as large as the calibre of the rubber tubing, thus assuring a more perfect aspiration of the material. The weight is also sufficient to permit rapid swallowing. Gravity assists its passage through the duodenum. As it can be left in the stomach without inconveniencing the patient, it is very suitable for the study of gastric juice over a long period.

The rubber tube is marked at three places with circles at distances of 18 inches, 25 inches and 32 inches from the tip, showing the distances from the mouth to the cardiac end, the fundus and the pyloric end of the stomach respectively.

Here again, the disadvantage is that the metal tip may separate from the tube.

Ryle's tube—This is a modification of the Rehfsuss tube. It consists of a small bore rubber tube of the diameter of a catheter No. 8 size. It has one blind end into which a small oval metal bulb (consisting of lead) is inserted. The bulb is thus completely covered by rubber. It acts as the weight. Just above it, there are two fairly large perforations in the rubber tube. The tube is marked with circles at distances of 16 inches, 22.5 inches, 28 inches and 32 inches, indicating the distance from the teeth to the cardiac end, the fundus, the pyloric end, and the duodenum respectively. The open end of the tube fits readily with the nozzle of a 20 c.c. record syringe by which the gastric contents are aspirated very easily.

The advantages claimed for the Ryle's tube are—

- 1 That it is more easily swallowed and withdrawn than a tube with a metal end.
- 2 That, as the perforations are in an elastic rubber wall, blockage with the mucus plugs is generally avoided, and, if it occurs, it can be easily removed by a syringe full of air.
- 3 That there is no possibility of the end becoming detached.
- 4 That there is less likelihood of trauma to the gastric mucosa than with a metal bulb.

In our experiments, Ryle's tube with a 20 c.c. record syringe was used. This proved quite satisfactory. The tube was sterilized by boiling, which process also softened the tube. The tip was dipped in glycerine before putting

in the mouth. This lubrication facilitated swallowing. The patient was directed to throw his head back, keep the lips shut, and breathe through the nose, while the tube was swallowed bit by bit. Care is taken that the saliva is not swallowed. This should be spat out into a beaker. As a rule not much difficulty is experienced in swallowing the tube. In the hospital class of patient, confidence can be gained by persuasion, and by the physician himself swallowing the tube in the presence of the patient.

EXPERIMENTAL

The present investigation was undertaken with the object of —

- (a) Ascertaining the composition of normal gastric juice in Indians,
- (b) Finding a suitable standard test meal for Indians for purposes of gastric analysis, and
- (c) Studying the response of the stomach to the test meal.

From the literature available it appears that no attempt has so far been made to study either the composition of normal gastric juice of Indians, or their gastric response to a standard test meal.

It is quite possible that these normal data for Indians are not quite the same as for Europeans and Americans, from whom the existing data have been obtained, as there are important differences of climate, diet, etc. The Indians, as a rule, live on a diet consisting largely of vegetables, while the inhabitants of Europe and America take meat habitually every day. It is also a question, if climate has any influence over the character of the digestive juices, especially when one compares the tropical heat of India with the mild climate of the European countries, where experimental work of this kind has been largely done.

We performed our experiments on normal subjects, who were all male adults varying in age between 18 and 43 years. Some of them were medical students, and some members of the staff of this laboratory. As the investigations necessitated the introduction of the stomach tube, the response from the volunteers was not encouraging. Later on, as their confidence was gained, the procedure did not appear so unksome, and more of them came forward for the purpose of gastric analysis.

The plan of the investigation was as follows —

- 1 The subject, whose gastric juice was to be analysed, was asked to take no food at night, except a glass of milk, and two charcoal biscuits at about 9 P.M. The following morning at about 8-30 or 9 A.M. before any food or drink was taken, the tube (Ryle's) was introduced and the stomach contents completely aspirated by means of a 20 c.c. record syringe. This specimen of resting juice was set aside for analysis.
- 2 While the tube was still in the stomach the person was made to swallow the test meal.

- 3 At intervals of 15 minutes after the test meal, a few c.c. of the gastric contents were aspirated with the record syringe and analysed. Specimens were withdrawn up to about two and a half hours after the test meal, or earlier if the stomach became empty.

The age, sex, and diet of each individual were recorded. They were all roughly classified into 'vegetarians' and 'non-vegetarians'. This classification is not an easy matter. Nearly all the individuals, vegetarians and non-vegetarians, on whom these observations were made, live on a diet composed chiefly of vegetables. Some take meat occasionally, and even those who profess to take a mixed diet of meat and vegetables, do not take meat as often as the Europeans. Therefore we consider that a rigid classification of Indians into 'vegetarians' and 'non-vegetarians' is not feasible. For purposes of comparison we classified all those who never touch meat as 'vegetarians' and the others who combine vegetables with meat occasionally or habitually as 'non-vegetarians'.

The vegetarian diet in this part of India consists of rice, wheat, dal, potatoes, peas, beans, aubergines, various green vegetables, sweets, milk, curd and other milk preparations, bananas, oranges, mangoes, and other fruits. Vegetables are cooked in ground-nut oil, or til-seed oil, but not as a rule in ghee (clarified butter) as is done in other parts of India. Rice is the staple article of diet and not wheat. The variety of meat taken is fish, eggs, mutton and chicken.

The results of the investigations may be considered under the following headings —

- 1 The resting gastric juice
- 2 The test meal
- 3 The response of gastric secretion to the test meal

I The resting gastric juice

A couple of charcoal biscuits and a glass of milk having been taken the previous night, all the resting gastric juice was withdrawn by means of Ryle's tube and a 20 c.c. record syringe the following morning. Its quantity, colour and smell were noted, and the presence of mucus, charcoal particles, blood, bile, and lactic acid looked for. Then the juice was filtered. Owing to the presence of mucus, filtration of the gastric juice is always a slow process. In some cases, we strained it through a piece of fine muslin, but the most satisfactory method in our experience is to centrifuge it. A combination of centrifugalization with filtration was also practised. On the whole we found that by centrifuging a clear enough specimen was obtained for purposes of analysis.

The analysis was carried out according to the method of Rehfuess. The amount of free hydrochloric acid was estimated by titration with N/10 NaOH, using Toepfer's reagent as the indicator. To this subsequently phenolphthalein was added, and titration continued to ascertain the total acidity. The end-point, in these titrations, is of course very sharp.

TABLE I

No	Name	Age	Diet	Total quantity in cc	Colour	Smell	Mucus	Charcoal particles	Starch	Blood	Bile	Lactic acid	FREE HCl		TOTAL ACIDITY	
													cc of N/10 NaOH	HCl %	cc of N/10 NaOH	HCl %
1	B P D	24	Mixed	12.5	Mucoid	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	9.0	0.03285
2	U K R	22	"	40.0	"	"	"	"	"	"	"	"	48.5	0.177	62.0	0.2265
3	H S P	31	"	8.0	"	"	"	"	"	"	"	"	27.5	0.10	42.5	0.155
4	M H M	20	"	40.0	Pale green	"	"	"	"	"	Present	"	28.0	0.102	48.0	0.175
5	P C M	22	"	35.0	Mucoid	"	"	"	"	"	Absent	"	30.0	0.109	48.0	0.175
6	B R	21	"	35.0	Yellowish	"	"	"	"	"	Present	"	24.0	0.0876	46.0	0.168
7	V B A	28	"	25.0	Mucoid	"	"	"	"	"	Absent	"	Absent	Absent	5.0	0.018
8	J R	24	"	15.0	"	"	"	"	"	"	"	"	5.0	0.0182	90.0	0.3285
9	J P A	24	"	6.0	"	"	"	"	"	"	"	"	Absent	Absent	7.5	0.0273
10	A L	21	"	34.0	"	"	"	"	"	"	"	"	6.25	0.0228	26.25	0.0958
11	D C	26	"	11.0	"	"	"	"	"	"	"	"	Absent	Absent	17.5	0.0638
12	J D D	31	"	8.0	"	"	"	"	"	"	"	"	"	"	7.5	0.0264
13	A A	18	"	71.0	"	"	"	"	"	"	"	"	3.75	0.0137	28.75	0.1049
14	A D	50	"	34.0	"	"	"	"	"	"	"	"	Absent	Absent	4.0	0.0146

15	J M	25	"	16.0	"	"	"	"	"	"	"	"	"	12.5	0.045	27.5	0.100
16	B N	43	"	13.0	"	"	"	"	"	"	"	"	"	20.0	0.073	35.0	0.1277
17	S L B	39	"	53.0	"	"	"	"	"	"	"	"	"	Traces only	Traces only	6.0	0.0219
18	J B A	22	Vegetarian	25.0	"	"	"	"	"	"	"	"	"	7.5	0.0274	22.5	0.0821
19	B S K	21	"	40.0	Yellowish	"	"	"	"	"	"	"	Present	2.5	0.0091	20.0	0.073
20	F K S	20	"	2.7	Mucoid	"	"	"	"	"	"	"	Absent	20.0	0.073	35.5	0.1295
21	L R M	30	"	10.0	"	"	"	"	"	"	"	"	"	Absent	Absent	5.0	0.01825
22	N G L	20	"	8.0	"	"	"	"	"	"	"	"	"	"	"	5.0	0.01825
23	K V M	24	"	30.0	Yellowish	"	"	"	"	"	"	"	Present	58.0	0.217	72.0	0.2628
24	M G T	19	"	35.0	Mucoid	"	"	"	"	"	"	"	Absent	30.0	0.109	47.0	0.171
25	S G T	23	"	14.0	"	"	"	"	"	"	"	"	"	19.5	0.07117	31.6	0.1151
26	J G P	19	"	60.0	Yellowish	"	"	"	"	"	"	"	Present	11.1	0.0116	29.0	0.1058
27	P P V	22	"	36.0	Mucoid	"	"	"	"	"	"	"	Absent	27.5	0.100	52.5	0.191
28	R H V		"	14.0	"	"	"	"	"	"	"	"	"	5.0	0.0182	26.25	0.0958
29	R D P	25	"	110.0	"	"	"	"	"	"	"	"	"	18.0	0.0657	29.0	0.106
30	B B	20	"	12.0	"	"	"	"	"	"	"	"	"	20.0	0.073	12.75	0.1560

In all, 30 normal individuals were examined. Of these 13 were classified as 'vegetarians' and 17 as 'non-vegetarians'. The results of analysis are given in Table I.

A consideration of the above table shows the following —

It will be seen that in the table the first 17 observations are on individuals whose diet is *mixed* or non-vegetarian, while the remaining 13 (Nos 18–30) are vegetarians.

1. *Total quantity of resting juice*

(a) This varies from 2.7 cc to 110.0 cc, the average quantity for all the 30 individuals being 28.473 cc.

(b) For 17 non-vegetarians, the quantities are —

Minimum—6.0 cc

Maximum—71.0 cc

Average—26.85 cc

(c) For 13 vegetarians, the quantities are —

Minimum—2.7 cc

Maximum—110.0 cc

Average—30.5 cc

There appears to be a great deal of variation in the amount of resting juice in different individuals. The variations would appear to be greater amongst the vegetarians. Although the average quantity of resting juice for all the 30 individuals is 28.473 cc, 20 of them (2/3 of the total number) had a figure varying between 11 cc and 40 cc, while 9 had a figure between 31 cc and 40 cc. The following table shows the total quantity figures distributed in decades cc's —

TABLE II

Quantity in cc	Non-vegetarians (17)	Vegetarians (13)	Total
1 to 10	3	3	6
11 to 20	5	3	8
21 to 30	1	2	3
31 to 40	6	3	9
41 to 50	0	0	0
51 to 60	1	1	2
61 to 70	0	0	0
71 to 80	1	0	1
81 to 90	0	0	0
90 to 100	0	0	0
101 to 110	0	1	1

It would appear that the amount above 60 c.c. is rare

2 *Colour and bile*—The colour in the majority of cases (24) was mucoid, slightly whitish, clear or opalescent. In six samples the colour varied from pale yellow to green. This was due to the presence of bile. The distribution of these 6 samples in the series was as follows —

	Number of specimens containing bile
Out of 17 non-vegetarians	3
Out of 13 vegetarians	3

It was observed that the colour of 'vegetarians' specimen was comparatively paler and darker. Thus bile was present in 20 per cent of all cases.

3 *Smell*—This was absent throughout. None of the specimens had any odour of putrefaction.

4 *Mucus*—This was present in all the specimens. The quantity varied considerably. One specimen contained a large amount of mucus, so much so, that the juice was aspirated by the syringe with much difficulty.

5 *Charcoal particles*—These were absent in all the specimens in this series, showing that the stomach had completely emptied itself overnight.

6 *Starch, blood and lactic acid*—These were absent in all the specimens.

7 *Free hydrochloric acid*—(a) There were 8 cases out of 30 in which free hydrochloric acid was completely absent, and one case in which it was present in traces only. Thus in about 27 per cent of this series, there was absence of free hydrochloric acid. The distribution of these nine cases was as follows —

	Specimens without free hydrochloric acid
Out of 17 non-vegetarians	7
Out of 13 vegetarians	2

(b) In the series of 30 individuals, thus, there were 21 containing free hydrochloric acid. The amount varied considerably, the distribution being as follows —

TABLE III

Observation series	MINIMUM AMOUNT OF FREE HCl		MAXIMUM AMOUNT OF FREE HCl		AVERAGE AMOUNT OF FREE HCl	
	c.c. of N/10 NaOH	HCl per cent	c.c. of N/10 NaOH	HCl per cent	c.c. of N/10 NaOH	HCl per cent
Total cases (21)	2.5	0.0091	58.0	0.217	20.23	0.07384
Non-vegetarians (10)	3.75	0.0137	48.5	0.177	20.55	0.0750
Vegetarians (11)	2.5	0.0091	58.0	0.217	19.94	0.0728

(c) Taking all the cases, including those containing free hydrochloric acid and those without, the *average amount of free hydrochloric acid* was as follows —

	cc of N/10 NaOH	HCl per cent
Total cases (30)	14.160	0.0517
Non-vegetarians (17)	12.09	0.044
Vegetarians (13)	16.87	0.0616

8 *Total acidity*—This also shows considerable individual variations, as is apparent from the following table —

TABLE IV

Observation series	MINIMUM TOTAL ACIDITY		MAXIMUM TOTAL ACIDITY		AVERAGE TOTAL ACIDITY	
	cc of N/10 NaOH	HCl per cent	cc of N/10 NaOH	HCl per cent	cc of N/10 NaOH	HCl per cent
Total cases (30)	5.0	0.018	90.0	0.3285	30.95	0.113
Non-vegetarians (17)	5.0	0.018	90.0	0.3285	30.02	0.110
Vegetarians (13)	5.0	0.018	72.0	0.2628	32.16	0.117

II Variations in the composition of the resting gastric juice in the same individual

We made repeated observations on the same individual on different days to see the variations that occurred in the composition and character of the gastric juice. The actual data obtained from 11 persons are as follows —

1 B S K, age 21 Vegetarian

	First observation	Second observation
Quantity	40.0 cc	70.0 cc
Colour	Yellow	Deep green
Smell	Nil	Slightly sour
Mucus	Present	Present
Charcoal particles	Nil	Nil
Starch	Nil	Nil
Blood	Nil	Nil
Bile	Present	Present
Lactic acid	Nil	Nil
Free HCl	25 cc N/10 NaOH or 0.009% HCl	10 cc N/10 NaOH or 0.036% HCl
Total acidity	20 cc N/10 NaOH or 0.073% HCl	30 cc N/10 NaOH or 0.109% HCl

2 K V M, age 24 Vegetarian

	First observation	Second observation
Quantity	1050 cc	300 cc
Colour	Mucoid	Pale yellow
Smell	Nil	Nil
Mucus	Present	Present
Charcoal particles	Nil	Nil
Starch	Nil	Nil
Blood	Nil	Nil
Bile	Nil	Present
Lactic acid	Nil	Nil
Free HCl	5 cc N/10 NaOH or 0.01825% HCl	110 cc N/10 NaOH or 0.0401% HCl
Total acidity	58 cc N/10 NaOH or 0.217% HCl	720 cc N/10 NaOH or 0.2628% HCl

3 M G T, age 19 Vegetarian

	First observation	Second observation
Quantity	350 cc	670 cc
Colour	Mucoid	Pale yellow
Smell	Nil	Nil
Mucus	Present	Present
Charcoal particles	Nil	Nil
Starch	Nil	Nil
Blood	Nil	Nil
Bile	Absent	Present
Lactic acid	Nil	Nil
Free HCl	300 cc N/10 NaOH or 0.1095% HCl	562 cc N/10 NaOH or 0.205% HCl
Total acidity	470 cc N/10 NaOH or 0.171% HCl	712 cc N/10 NaOH or 0.2599% HCl

4 S G T, age 23 Vegetarian

	First observation	Second observation
Quantity	140 cc	550 cc
Colour	Mucoid	Mucoid
Smell	Nil	Nil
Mucus	Present	Present
Charcoal particles	Nil	Nil
Starch	Nil	Nil
Blood	Nil	Nil
Bile	Nil	Nil
Lactic acid	Nil	Nil
Free HCl	19.5 cc N/10 NaOH or 0.07117% HCl	43.2 cc N/10 NaOH or 0.15766% HCl
Total acidity	31.6 cc N/10 NaOH or 0.1154% HCl	56.4 cc N/10 NaOH or 0.2058% HCl

5 J G P, age 19 Vegetarian

	First observation	Second observation
Quantity	60.0 cc	80.0 cc
Colour	Yellow	Greenish
Smell	Nil	Nil
Mucus	Present	Present
Charcoal particles	Nil	Nil
Starch	Nil	Nil
Blood	Nil	Nil
Bile	Present	Present
Lactic acid	Nil	Nil
Free HCl	11.4 cc N/10 NaOH or 0.04161% HCl	24.4 cc N/10 NaOH or 0.0890% HCl
Total acidity	29.0 cc N/10 NaOH or 0.1058% HCl	38.0 cc N/10 NaOH or 0.14016% HCl

6 R D P, age 25 Vegetarian

	First observation	Second observation
Quantity	110.0 cc	47.0 cc
Colour	Mucoid	Mucoid
Smell	Nil	Nil
Mucus	Present	Present
Charcoal particles	Nil	Nil
Starch	Nil	Nil
Blood	Nil	Nil
Bile	Nil	Nil
Lactic acid	Nil	Nil
Free HCl	18.0 cc N/10 NaOH or 0.0657% HCl	10 cc N/10 NaOH or 0.0146% HCl
Total acidity	29.0 cc N/10 NaOH or 0.106% HCl	13.4 cc N/10 NaOH or 0.049% HCl

7 J B A, age 22 Vegetarian

	First observation	Second observation	Third observation
Quantity	92 cc	25.0 cc	10.5 cc
Colour	Mucoid	Mucoid	Dark green
Smell	Nil	Nil	Nil
Mucus	Present	Present	Present
Charcoal particles	Nil	Nil	Present
Starch	Nil	Nil	Nil
Blood	Nil	Nil	Nil
Bile	Nil	Nil	Present
Lactic acid	Trace only	Nil	Present
Free HCl	Absent	7.5 cc N/10 NaOH or 0.0274% HCl	22.5 cc N/10 NaOH or 0.0821% HCl
Total acidity	2.6 cc N/10 NaOH or 0.0095% HCl	22.5 cc N/10 NaOH or 0.0821% HCl	50.0 cc N/10 NaOH or 0.1825% HCl

8 B P D, age 24 Non-vegetarian

	First observation	Second observation
Quantity	125 cc	33.5 cc
Colour	Mucoid	Mucoid
Smell	Nil	Nil
Mucus	Present	Present
Charcoal particles	Nil	Nil
Starch	Nil	Nil
Blood	Nil	Nil
Bile	Nil	Nil
Lactic acid	Nil	Nil
Free HCl	Absent	Absent
Total acidity	90 cc N/10 NaOH or 0.03285% HCl	12.5 cc N/10 NaOH or 0.0456% HCl

9 U K R, age 22 Non-vegetarian

	First observation	Second observation
Quantity	400 cc	470 cc
Colour	Mucoid	Yellowish
Smell	Nil	Nil
Mucus	Present	Present
Charcoal particles	Nil	Nil
Starch	Nil	Nil
Blood	Nil	Nil
Bile	Nil	Nil
Lactic acid	Nil	Nil
Free HCl	48.5 cc N/10 NaOH or 0.177% HCl	70.0 cc N/10 NaOH or 0.255% HCl
Total acidity	62.0 cc N/10 NaOH or 0.2263% HCl	85.0 cc N/10 NaOH or 0.310% HCl

10 S L B, age 39 Non-vegetarian

	First observation	Second observation
Quantity	53.0 cc	48.0 cc
Colour	Mucoid	Mucoid
Smell	Nil	Nil
Mucus	Nil	Nil
Charcoal particles	Nil	Nil
Starch	Nil	Nil
Blood	Nil	Nil
Bile	Present	Nil
Lactic acid	Nil	Nil
Free HCl	Traces only	Absent
Total acidity	6.0 cc N/10 NaOH or 0.0219% HCl	13.6 cc N/10 NaOH or 0.0496% HCl

Bile was present in the last portion of the sample drawn with the syringe

11 H S P, age 31 Non-vegetarian

	First observation	Second observation	Third observation	Fourth observation
Quantity	8.0 cc	5.0 cc	13.5 cc	10.2 cc
Colour	Mucoid	Mucoid	Mucoid	Mucoid
Smell	Nil	Nil	Nil	Nil
Mucus	Present	Present	Present	Present
Charcoal particles	Nil	Nil	Nil	Nil
Starch	Nil	Nil	Nil	Nil
Blood	Nil	Nil	Nil	Nil
Bile	Nil	Nil	Nil	Nil
Lactic acid	Nil	Nil	Nil	Nil
Free HCl	27.5 cc N/10 NaOH or 0.100% HCl	5.0 cc N/10 NaOH or 0.018% HCl	3.25 cc N/10 NaOH or 0.0118% HCl	36.5 cc N/10 NaOH or 0.1332% HCl
Total acidity	42.5 cc N/10 NaOH or 0.155% HCl	16.0 cc N/10 NaOH or 0.058% HCl	9.0 cc N/10 NaOH or 0.0328% HCl	48.0 cc N/10 NaOH or 0.177% HCl

It will be seen that observations were repeated on 11 cases more than once. Of these two observations were made on 9, three on one, and four on one. The first seven are vegetarians and the last four non-vegetarians. An examination of the above analysis shows that certain features of the normal gastric juice remain remarkably constant. Thus it is free from smell, charcoal particles, starch, blood, lactic acid. Mucus seems to be a normal constituent of gastric juice. Absence of charcoal particles indicates complete emptying overnight. They were absent on all occasions except one, namely in case number 7 J B A, when the juice was dark green in colour, had a sour smell and contained traces of lactic acid. This was the only exception to the rule. It indicates incomplete emptying of the stomach and excessive fermentation. The chief variations are in regard to quantity, presence of bile, free HCl, and total acidity.

Quantity—This appears to vary to a certain extent in the same individual from day to day. This is well brought about by the data given above. The fluctuations seem to be greater in the case of vegetarians than non-vegetarians.

Bile—The presence of bile is a variable factor.

(i) It was completely absent in four cases, namely —

Case number 4 S G T

Case number 6 R D P

Case number 8 B P D

Case number 11 H S P

Of these the first two were vegetarians and the last two non-vegetarians.

(ii) In the following five cases it was present on one occasion and absent on another —

Case number 2 K V M

Case number 3 M G T

Case number 7 J B A

Case number 9 U K R

Case number 10 S L B (Here it was present in the last portion of the sample drawn with the syringe on the first occasion)

The first three were vegetarians and the last two non-vegetarians.

(iii) In the following two cases it was present on all occasions —

Case number 1 B S K

Case number 5 J G P

Both were vegetarians.

Colour—The colour varies according to the presence or absence of bile and also according to the amount of bile present.

The presence of bile indicates regurgitation of duodenal contents into the stomach. It may be a natural attempt to neutralize the acid juice. It is interesting to note that in case number 10 S L B, on one occasion, although the first part of the juice drawn out was perfectly clear, and free from bile, the latter part contained bile and was pale greenish in colour.

Free HCl and total acidity—This showed definite fluctuations. There are cases amongst non-vegetarians, number 8 B P D and number 10 S L B, when there was complete absence of free HCl on each occasion the fasting gastric juice was analysed. Amongst the vegetarians, the gastric juice of case number 7 J B A showed no free acidity on one occasion on the other two occasions when the juice was analysed, free HCl was present.

It was also noted that whenever the bile was present in the fasting juice, the amount of free HCl and Total Acidity were higher, and the quantity of juice also was, roughly speaking, greater. This is evident from the following table.

TABLE V

Name	Number of observations	Quantity of gastric juice in cc	Free HCl cc N/10 NaOH	Total acidity cc N/10 NaOH	Colour of gastric juice and presence of bile	Diet
J B A	I	9.2	Absent	2.6	Clear No bile	Vegetarian
	II	25.0	7.5	22.5	Clear No bile	
	III	16.5	22.5	50.0	Juice yellow Bile present	
K V M	I	105.0	5.0	11.0	Clear No bile	Vegetarian
	II	30.0	58.0	72.0	Yellow Bile present	
M G T	I	35.0	30.0	47.0	Clear No bile	Vegetarian
	II	67.0	56.2	71.2	Yellow Bile present	
B S K	I	40.0	2.5	20.0	Yellow Bile present	Vegetarian
	II	70.0	10.0	30.0	Green Bile present	
J G P	I	60.0	11.4	29.0	Yellow Bile present	Vegetarian
	II	80.0	24.4	38.0	Green Bile present	
U K R	I	40.0	48.5	62.0	Clear No bile	Non-vegetarian
	II	47.0	70.0	85.0	Yellow Bile present	

In the above table it will be seen that when in the same individual the gastric juice is yellow on one occasion and greenish at another, the amount of acidity is higher in the green sample, which presumably contains a greater amount of bile.

It was also observed, that in cases in which the free HCl is very low or completely absent, the juice was as a rule clear.

DISCUSSION

The glands in the mucous membrane of the stomach, like any other glands in the body, produce a continuous secretion. But the amount, rate and composition of the secretion varies according to the mode of stimulation under various physiological and pathological conditions. The composition of the secretion is also altered by admixture with saliva and duodenal contents. In the juice of the 'fasting' stomach a certain amount of saliva is usually present, but the duodenal juices are as a rule absent. If the 'fasting' secretion is unusually rapid, it may have the same composition as that of the pure gastric juice produced during the digestion of a meal.

The quantity of juice in the 'fasting' stomach varies considerably in health. The following observations may be quoted —

- 1 Verhagen reports as high as 50 c c, with a range of 10 to 25 c c
- 2 Moitz reports 24 to 64 c c
- 3 Rehfuess, Berghelm and Hawk find 30 to 180 c c
- 4 Fowler and Zentmire found an average of 50 c c in 90 healthy women
- 5 Carlson reports that the average of several hundred observations on three gastric fistula cases (2 adult men and a girl of twelve, all having cicatricial stenosis of the pylorus) is 30 c c, with variations of 5 c c to 120 c c

He has found normal subjects with the stomach literally empty in the morning before breakfast. He has also noted that the contents of the stomach are greater in the morning before breakfast than at noon before lunch. It was also greater in the summer months than in the winter months. Such variations, according to him, are probably related to the gastric tonus and mobility rather than to the rate of continuous secretion.

6 Ryle found that the quantity varied from 10 to 150 c c, with an average of 54 c c. Bile was present in 40 per cent of fasting specimens. In his own gastric juice, the quantities varied from a few c c obtained with difficulty to 20 or 30 c c obtained with ease.

The acidity also varies considerably from zero to full acidity found at the height of gastric digestion.

(a) For his three gastric fistula cases, Carlson gives the following figures

	FREE ACIDITY, PER CENT			TOTAL ACIDITY, PER CENT		
	Low	High	Average	Low	High	Average
Mr V	0.10	0.35	0.18	0.15	0.40	0.23
Mr E	0.09	0.36	0.20	0.20	0.42	0.25
Miss C	0.08	0.40	0.13	0.13	0.45	0.26

(b) Ryle found the range of free acidity from 0 to 60 per cent N/10 NaOH in all his cases, and from 0 to 22 in the eighty cases providing the figures for his standard chart

The free acidity in his own juice varied from 0 to 22 and the total acidity from 4 to 38, with a difference between free and total acidity ranging from 10 to 15. Bile in his fasting juice was present or absent with equal frequency, and sometimes absent in the first syringeful and present in the second, suggesting that either aspiration or automatic reflux from the duodenum had occurred. It may be noted that the same phenomenon was noted in the juice of one of us (S L B)

From the observations quoted above it would seem that our own data fall well within the accepted normal standards. Our own findings may be summarized as follows —

SUMMARY

1 The 'fasting' gastric juice of 30 normal male adult Indians was examined. Seventeen of the subjects were classified as non-vegetarians and 13 as vegetarians.

2 The quantity of the juice varied from 2.7 c.c. to 110 c.c., the average being 28.473 c.c. The average quantity was a little higher in the case of vegetarians than non-vegetarians.

3 Bile was present in 20 per cent of all cases. Mucus was present in all. Charcoal particles, starch, blood and lactic acid were absent.

4 In 27 per cent of the cases free HCl was absent. In those in whom it was present, it varied from 2.5 c.c. N/10 NaOH (0.0091 per cent HCl) to 58 c.c. N/10 NaOH (0.217 per cent HCl), the average being 20.23 c.c. N/10 NaOH (0.07384 per cent HCl). There was no appreciable difference in the amount of free HCl between the non-vegetarians and vegetarians.

5 Total acidity varied from 5.0 c.c. N/10 NaOH (0.018 per cent HCl) to 90.0 c.c. N/10 NaOH (0.3285 per cent HCl), the average being 30.95 c.c. N/10 NaOH (0.113 per cent HCl). There was no appreciable difference between the non-vegetarians and vegetarians in this regard.

6 In 11 cases, observations were repeated more than once. This showed that while the absence of smell, charcoal particles, starch, blood and lactic acid was a constant feature (with one exception in regard to the charcoal particles and lactic acid), there was marked variation in regard to the quantity of the juice, the presence of bile, free HCl and total acidity. Actual data are given indicating these variations.

7 It was observed in many cases, that when bile was present in the juice, the amount of free HCl and total acidity were relatively higher, indicating that regurgitation of duodenal contents had taken place, presumably with the object of reducing the acidity of the gastric juice.

These investigations were carried out with the help of a grant from the Indian Research Fund Association, to which we offer our best thanks.

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‘LACTO-CHLORAL’ A NEW CLEARING AND MOUNTING
MEDIUM FOR THE RAPID OBSERVATIONS OF THE
MICROSCOPICAL STRUCTURES OF SMALL
INSECTS

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[Received for publication, March 30, 1931]

OWING to the growing importance of internal structures as the bases of the latest modes of classification of insects, the value of a clearing agent that will act quickly by its rapid permeation through the exoskeleton of arthropods without any appreciable distortion of the lightly chitinized structures and thereby aid in the quick examination of a series of specimens within a reasonable period of time, cannot be gainsaid. The usual caustic potash method of clearing the arthropod exoskeleton is a long and tedious one, and although the lengthy procedure can be appreciably shortened by the passage of the object through a neutralizing agent, such as acetic acid, yet, owing to the passage of the object primarily for several hours through a strong alkali and secondarily through aqueous medium or an acid, a distortion and thereby a misrepresentation of certain lightly chitinized structures, which are generally of considerable importance to the morphological systematists of the present day, is wellnigh inevitable. Amann's lacto-phenol is decidedly an improved innovation in that its action is comparatively quicker, as no filtering is at all necessary, it can be prepared in any laboratory and is a suitable reagent for field or outdoor work where the time factor is of considerable importance. The only defect of the medium is that it is slightly amber coloured and this sometimes renders observations difficult during the process of clearing. It therefore devolved on me to find out by trial a medium of uniform fluidity composed of perfectly

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miscible liquids that would act more quickly than most of the known reagents and would at the same time be sufficiently transparent to make microscopical examination of the object possible even during the process of clearing. Reagents in the following proportions were used for clearing the buccal-armature of the flies of the *minutus* group of the genus *Phlebotomus*

Chloral hydrate	0.5 g
Distilled water	1 c c
Glycerine	1 c c
Lactic acid (extra pure)	2 c c
Glacial acetic acid	2-4 minims
Formol	0.5 c c

The medium is easy to prepare and no filtering or heating is necessary since the fluids are perfectly miscible. When pure ingredients are used the resultant fluid has the advantage over lactophenol in that it is perfectly colourless and transparent. The ingredients should be mixed in the order given above and it is advisable to use fresh medium each time. The following table will show the comparative time* taken for complete penetration or clearing in 3 different clearing media —

	Caustic potash	Lacto phenol	Lacto chloral
Average time taken to clear the head of a <i>Phlebotomus</i>	Overnight	30 minutes or over (at room temperature)	10 to 15 minutes (at room temperature)
Penetration as determined by floating or sinking of a whole insect	Floating even after overnight's treatment. Capillary action on the hairs of the body	Only partially submerged after 20 minutes' treatment. Less capillary action on the hairs of the body	Completely submerged after 20 minutes' treatment. Very little capillary action on the hairs of the body

Method of clearing — Some clearing medium is dropped with a pipette on the cell of an excavated slide and the entire object or its part as the case may be to be cleared is carefully dropped into the medium and the cell covered with a cover glass. Immediately the clearing of the parts begins to take place which should be watched through a binocular microscope. The medium may conveniently be used as a permanent mountant with satisfactory results. A Nematode and a sandfly larva mounted in this medium more than a month back although sufficiently cleared have kept their original colour.

* The time taken varies directly with the bulk of the object

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THE DISTRIBUTION OF ANTIMONY IN THE BODY ORGANS

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[Received for publication, April 2, 1931]

IN a recent paper by two of the present writers dealing with the rate of excretion of antimony in the urine after administration as a therapeutic measure in the treatment of kala-azar, we gave our results with reference to two antimony preparations, a pentavalent compound, Bayer 693B, di-ethylamine para-amino-phenyl stibiate, and a trivalent salt, sodium antimony tartarate, and we showed the great difference between the two preparations as regards their rate of excretion

In the investigations reported in this paper we have attempted to estimate the actual distribution of antimony in the various tissues and organs of the body after administration in sub-lethal doses. The compound used was Bayer 693B, the experiments being carried out on monkeys. The object of carrying out these experiments was to obtain some information as to whether the antimony is deposited or held up in any of the various organs, in the hope that some light might be thrown on the very complex problem as to why antimony should act as a curative agent in kala-azar.

It has been shown by one of the writers (L. E. N.) and by other workers that the therapeutically active pentavalent compounds of antimony in concentrations ten times greater than they could ever be present in the blood when given in therapeutic doses have no action whatsoever on the cultural forms of leishmania, it seems impossible therefore to consider the action as a direct one. On the analogy of the arsenic compounds it has been assumed that interaction between certain body cells and the antimony produces a highly toxic substance which is inimical to the leishmania. For this theory no support has been gained from *in vitro* experiments and furthermore it seems unreasonable to accept it when there is an equally good theory based on purely biological grounds, i.e., the stimulation of the reticulo-endothelial system in such a way that its units are capable of destroying the parasite. It was, therefore, of interest to see if we should find larger amounts of antimony in those organs particularly rich in cells of the reticulo-endothelial type.

METHOD OF ANALYSIS EMPLOYED

This closely resembles the method employed by us (Boyd and Roy, 1929) when dealing with urine. The organs are weighed and cut into very fine pieces, then heated in a porcelain basin on a water bath to get rid of moisture and to facilitate further breaking up which is done with a thick glass-rod. The mash is then carefully transferred to a 500 c.c. Kjeldahl flask and the basin treated with two successive washings of 5 c.c. of warm concentrated hydrochloric acid (Sp. Gr. 1.124 to 1.126) and several successive small amounts of warm water, all of which are transferred to the Kjeldahl flask. About 2 grammes of potassium chlorate are then added and the flask is heated over a small flame with frequent gentle agitations of the contents. Potassium chlorate and hydrochloric acid in the proportion of 1 gramme to 4 c.c. of HCl are added at half-hourly intervals until the content of the flask is converted into a pale yellow liquid with a layer of fatty matter floating on the surface. If at this stage the liquid darkens in colour on prolonged heating the process is incomplete and heating has to be continued with the addition of further small amounts of potassium chlorate and hydrochloric acid in the above-mentioned proportion until the liquid ceases to darken. This procedure removes the bulk of the organic matter. The further steps employed are identical with those described in the above-mentioned paper on the estimation of antimony in the urine.

The results of our experiments are shown in the following tables —

TABLE I

Weight of monkey 2,500 grammes Dose of '693' (41 per cent antimony) 0.295 gramme = 120.95 mg of metallic antimony, or 0.04835 mg per gramme of tissue, given intravenously, monkey killed 14 minutes later

Organs	Weight or volume grammes or cc	Actual Sb content found in milli- grams
Lungs	15.5	3.0
Heart	9.5	0.8
Brain	35.0	Trace
Bone	23.1	0.16
Blood	50.0	5.6
Intestinal tract and contents	216.0	0.18

TABLE II

Weight of monkey 2,570 grammes Dose of '693' 0.2 gramme = 82 mg of metallic antimony, or 0.0319 mg per gramme of tissue, given intravenously, monkey killed 13 minutes later

Organs	Weight or volume, grammes or cc	Actual Sb content found, in milli- grams
Lungs	12.1	1.2
Kidneys	14.6	1.3
Liver	85.0	0.9
Spleen	2.7	0.04
Heart	10.6	0.56
Brain	6.2	Nil
Bone	12.4	0.08
Pancreas	4.3	0.2
Heart blood	30.0	2.6
Blood	25.0	2.4

TABLE III

Weight of monkey 3,270 grammes Dose of '693' 0.282 gramme = 115.6 mg of metallic antimony, or 0.03535 mg per gramme of tissue, given intravenously, monkey killed 20 minutes later

Organs	Weight or volume, grammes or cc	Actual Sb content found, in milligrams
Lungs	21.0	2.4
Kidneys	14.5	7.5
Liver	73.0	1.5
Spleen	5.0	0.12
Heart	10.5	0.4

TABLE IV

Weight of monkey 2,700 grammes Dose of '693' 0.18 gramme = 73.8 mg of metallic antimony, or 0.02733 mg per gramme of tissue, given intravenously, monkey killed 20 minutes later

Organs	Weight or volume, grammes or cc	Actual Sb content found, in milligrams
Liver	72.5	1.0
Spleen	5.0	0.06

TABLE V

Weight of monkey 2,600 grammes A total dosage of 1.0 gramme of '693' was given in 0.1 doses on alternate days over a period of 3 weeks = 410 mg of metallic antimony, or 0.1577 mg per gramme of tissue, monkey killed 48 hours after last injection

Organs	Weight or volume, grammes or cc	Actual Sb content found, in milligrams
Lungs	19.5	Nil
Kidneys	19.5	2.0
Liver	81.0	6.5
Spleen	5.5	0.04
Heart	16.0	Nil
Blood	50.0	Nil

TABLE VI

Weight of monkey 3,700 grammes Dosage of '693' given by intravenous route, 1st day, 0.1 g, 4th day, 0.2 g, 6th day, 0.2 g, 8th day, 0.2 g, 10th day, 0.2 g, 12th day, 0.2 g, 14th day, 0.2 g, 17th day, 0.2 g, 19th day, 0.2 g, killed 48 hours after the last injection Total dosage of 1.7 grammes in 19 days = 697 mg of metallic antimony, or 0.1884 per gramme of tissue

Organs	Weight or volume grammes or cc	Actual Sb content found, in milli- grams
Spleen	3.1	6.08 mg
Kidneys	18.2	2.5
Heart	15.8	0.01
Liver	111.5	8.5
Lungs	34.0	6.16
Muscle	17.8	Nil
Brain	62.0	Nil
Long bones	20.6	Nil
Hair	11.5	0.12
Blood	50.0	Nil

Taking the individual experimental animals it is difficult to evaluate the actual amount of antimony per organ so that it can be visualized and to a certain extent compared as a check against experimental errors. To obtain this the results of the estimations are best regrouped, organ by organ, so as to show the antimony content per gramme of organ actually found on analysis, and compare this against what would be expected provided there had been even distribution of the drug throughout the body. The results regrouped in this manner are shown in Tables VII and VIII.

In order to obtain an idea of the relative distribution of antimony throughout the body we have compared the amount found in the various tissues, organs and fluids with the amount which each would contain had the antimony injected been distributed evenly throughout the body, thus we have expressed as milligrams per gramme of tissue and have referred to it as the 'normal quota'. It is unnecessary to point out that calculations based purely on a uniform distribution rate must be misleading. Where animal organs are concerned physical factors, such as the rate of the flow of blood through the various organs, must be taken into consideration.

TABLE VII.

Antimony content of organs or short tissue after single dose

Tissue or organ	Serial number of monkey	Time between injection and death	Antimony content per gramme of tissue	Expected antimony content pie-form distribution of Sb throughout all tissues of body, i.e., 'normal quota'	Whether antimony content greater or less than 'normal quota'	Proportion of antimony content to 'normal quota'
		Minutes	Milligrams			
Lung	I	15	0.190	0.050	+	3.8/1
	II	13	0.091	0.031	+	2.9/1
	III	20	0.114	0.035	+	3.2/1
Heart	I	15	0.084	0.050	+	1.7/1
	II	13	0.053	0.031	+	1.7/1
	III	20	0.038	0.035	+	1.1/1
Kidney	II	13	0.090	0.031	+	2.9/1
	III	20	0.510	0.035	++	14.3/1
Blood	I	15	0.110	0.050	+	2.2/1
	II	13	0.086	0.031	+	2.8/1
Brain	I	15	Trace only (in 35 grammes)	0.050	—	
	II	13	None detected (in 6.2 grammes)	0.031	—	
Bone	I	15	0.0068	0.0500	—	1/7.4
	II	13	0.0060	0.0310	—	1/5.2
Liver	II	13	0.010	0.031	—	1/3.1
	III	20	0.020	0.035	—	1/1.7
	IV	20	0.013	0.027	—	1/2.1
Spleen	II	13	0.015	0.031	—	1/2.1
	III	20	0.024	0.035	—	1/1.4
	IV	20	0.012	0.027	—	1/2.25
Intestinal tract and contents	I	15	0.0022	0.0500	—	1/22.7
Pancreas	II	13	0.046	0.031	+	1/5.1

TABLE VIII

Antimony content of organs 48 hours after last dose of course of injections

Organ or tissue	Serial number of monkey	Antimony content per gramme of tissue, in milligrams	Antimony content presuming even distribution in all organs, if there had been no excretion
Lungs	V	No trace (in 19 grammes)	0.158
	VI	0.0047	0.188
Heart	V	No trace (in 16 grammes)	0.158
	VI	0.0025	0.188
Kidney	V	0.102	0.158
	VI	0.136	0.188
Liver	V	0.08	0.158
	VI	0.076	0.188
Spleen	V	0.0072	0.158
	VI	0.023	0.188
Muscle	VI	No trace (in 17.8 grammes)	0.188
Brain	VI	No trace (in 62 grammes)	0.188
Bone	VI	No trace (in 20.6 grammes)	0.188
Hair	VI	0.01	0.188
Blood	V	No trace (in 50 cc)	0.158
	VI	No trace (in 50 cc)	0.188

In the animals killed shortly after a single injection (Table VII) the amount of antimony found in the blood is—taking the mean of the two readings—about 0.1 milligram per gramme of blood. Calculating that the blood is 1/15th of the body weight this means that only about one-sixth of the antimony originally injected into it is still in the blood, the other 5/6ths having been removed. However, the blood still contains about 2.5 times the normal quota.

The largest relative amount of antimony is to be found in the kidneys if we take the mean of the two readings. There is, in the case of the kidneys, considerable discrepancy between the two estimations. It is the only instance in this enquiry in which there is any marked discrepancy between two estimations. It is possible that there has been an error in technique, but at the same time there may be another explanation. Antimony is very rapidly excreted through this organ, after 20 minutes the tubules and even the pelvis of the kidney may have contained a considerable amount of antimony and, as unfortunately no special precautions were taken to wash out the kidney pelvis, this amount may or may not have escaped when the kidney was removed. The estimation in the case of No. 3 monkey's kidney was more than 14 times the

normal quota, whereas that of No 2 was only about 3 times. In the latter case the interval was only 13 minutes, if there is a delay in the commencement of excretion the difference in the interval between the time of injection and the death of the animal in the two cases might also to some extent account for the discrepancy in the results.

The amount found in the lungs is also relatively high. There is very little discrepancy amongst the three estimations, they vary between three and four times the normal quota. The presence of this relatively large amount of antimony in the lungs is quite easy to understand, as the whole of the antimony solution in a comparatively high state of concentration passes through the lung capillaries and a large amount of antimony is probably taken up during the first passage of the antimony solution through these organs before it is distributed over the much wider area of the systemic circulation.

The amount in the heart is again a little above the normal quota, this can also be explained on the grounds of the very efficient blood supply to this organ. The figure for the pancreas is slightly above the normal quota, but the estimation was only done in one case.

All the estimations—three in each case—for the liver and spleen approximate to one another very closely and are on the average about half that of the normal quota. Both these are highly vascular organs, it is therefore quite obvious that a very small quantity is taken up by the cells in the organs themselves, as a good deal of the antimony found can be accounted for by its presence in the blood which the organs must contain.

The long bones also contained a relatively small quantity—about one-sixth the normal quota—whereas the brain and intestinal tract contain only a trace. The relatively small amount present in the intestinal tract can to a certain extent be accounted for by the fact that the contents and the intestinal tract were weighed together but, even allowing for the fact that the intestinal contents probably contained no antimony at all, the figure for the tract itself must be exceedingly low, probably less than one-tenth the normal quota.

These figures do not suggest that any particular organ or system of cells in the body has any immediate affinity for the antimony. There would appear to be no indication that the reticulo-endothelial cells possess any such affinity,* as in both the spleen and liver, two organs rich in reticulo-endothelial cells, there is a comparatively small amount of antimony present.

* Recently, Brahmachari, Sen and Banerjee (1930) claim to have shown that when a leishmania-infected mouse is given an injection of finely divided antimony the latter is taken up by the leishmania-infected cells in the spleen. This is almost certainly true. Kala-azar is essentially a disease of the reticulo-endothelial system and it is, therefore, in these cells in the spleen, as well as in other parts of the body, that the parasites will be found. If a suspension of finely divided particles, whether they be of metallic antimony or of Indian ink, are injected into the blood stream in suitable doses the particles will be taken up by the reticulo-endothelial cells of the spleen whether these be leishmania-infected or not. The fate of the antimony noted by these workers appears to be dependent rather on its physical condition than on its chemical composition.

Forty-eight hours after the last administration of a course of antimony injections, the distribution in the tissues shows a very different picture. By this time a very large percentage of the antimony injected has been excreted. It is not possible to say exactly what proportion has been excreted, but from the fact that none could be detected in the blood, the muscles or the bones and that the liver and kidneys were the only organs to contain any appreciable quantity, it seems quite certain that the remaining antimony must amount to less than 10 per cent of the original amount injected, so that when reference is made to the 'normal quota' which is based on the total amount of antimony injected it must be remembered that this should be divided by at least 10 to get an idea of the relative quantity of antimony present in each organ, were there even distribution throughout all organs and tissues of the antimony still remaining in the body?

No antimony could be detected in 50 c.c. of the blood in either instance. The amount in the lungs was in one case not detectable and in the other little more than a trace. Whereas in the estimation shortly after the injection it was 3 to 4 times the normal quota, it is now only about a fortieth of this. In the heart also only a trace is now found. Proportionately the largest amount is still found in the kidneys, this is to be expected as the kidneys are the main channel for elimination from the body, as the writers have shown (Boyd and Roy, 1929). Although the rate at which excretion occurs falls rapidly, traces can still be found even as late as the 20th day.

The amount of antimony in the liver is the point of greatest interest, here there is relatively an enormous increase. Whereas in other organs and tissues there is a very marked decrease in the antimony per gramme of tissue, there is in the case of the liver—taking the mean of the readings—a five-fold increase. This is a definite indication that there is storage of the antimony in the liver. The antimony is not taken up immediately by the cells of this organ but is distributed widely in the body, and later carried into the bloodstream to the liver. Though this storage may occur in the Kupffer's cells there is not a loading of the cells of the general reticulo-endothelial system as little more than a trace is to be found in the lung and only small quantities in the spleen.

It is interesting to note that an appreciable stain was observed on the paper when the hair was tested, this is possibly another channel by which antimony is eliminated from the body.

CONCLUSIONS

From observations made by injecting a pentavalent compound of antimony, di-ethylamine para-amino-phenyl stibiate, a compound which has a marked therapeutic action in kala-azar, into monkeys, it does not appear that any particular organ or system has any marked affinity for the antimony. The immediate distribution of antimony would appear to be entirely dependent on

physical factors As we have shown elsewhere, the main channel of elimination of the antimony compound is through the kidneys Forty-eight hours after the last dose of a course of injections the bulk of the remaining antimony is found in the liver where it is possibly stored prior to being excreted, but there does not appear to be any general loading of the fixed reticulo-endothelial cells of the body with antimony

These experiments do not lend any support to the theory that antimony acts by stimulating the function of the reticulo-endothelial cells yet they in no way disprove this theory, as the reception of a stimulus need not necessarily be accompanied by storage of the stimulating substance, in fact it is more likely that the giving of the stimulus would result in a throwing off of the stimulant, on the other hand they lend definite support to our contention that the action of antimony is an indirect one

REFERENCE

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AN EPIDEMIOLOGICAL INVESTIGATION OF KALA-AZAR IN A RURAL AREA IN BENGAL

BY

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(An investigation under the Indian Research Fund Association)

[Received for publication April 2, 1931]

THE Kaoriapukku treatment centre is situated in a village in Bengal about five miles from Calcutta. Although this village is near Calcutta the conditions are not in any way similar to those of the suburbs of the city or of the large industrial areas in its neighbourhood. The conditions are, in fact, typically rural. The village itself is situated at the end of a cart road, about two miles from the main road, but most of the villages in the surveyed area are only approachable by footpath, and during the monsoon months when the rice fields are flooded some of them can only be reached by the aid of a boat. A canal (*khal*) runs through the area, this constitutes the main channel for the transportation of rice from the surrounding villages to Calcutta or to the rice mills which are situated between this area and Calcutta itself. In Kaoriapukku itself there is a Christian mission, under the London Missionary Society. This society has a boys' school and a girls' school, the former is managed by a European gentleman and his wife and the latter by two European ladies. It was the presence of this mission that determined our selection of this area for investigation as the missionaries have the confidence of the villagers of all communities in the district. Their sponsorship of our undertaking undoubtedly tided us over a period of prejudice, the unaided surmounting of which would have seriously interfered with our work during the first year. Furthermore, the mission placed a room at our disposal and supplied

us with a considerable amount of personal assistance, without the latter it would have been impossible for the one doctor to carry on the work on the scale necessary.

The great majority of the villagers are cultivators and work in the neighbouring rice fields, a very few are employed outside, mostly as mechanics by the Calcutta Tramway Company. They are Hindus, Mohammedans and Christians, the last-named of at least three denominations.

During the years previous to the establishment of this treatment centre a considerable amount of public attention had been directed towards kala-azar and numerous voluntarily-supported treatment centres were springing up all over Bengal, particularly in the vicinity of Calcutta. It was reported to us from patients in our out-patient department that not only were there a considerable number of cases in this area, but that the patients were unable to obtain treatment locally. This was an additional determining factor in our choice of site for our investigation as it would have been unsatisfactory had any extensive treatment of the inhabitants been previously undertaken, in the circumstances we were practically working on virgin soil. We visited the area and with the aid of the missionary-in-charge mapped out a certain area. We then let it be known that we would treat all patients suffering from kala-azar who lived within this area but that persons coming from outside this area would not be treated. In actual practice we usually gave a 'placebo' mixture to any of the latter class that attended, but we told them quite definitely that they must not come again. Naturally enough instances occurred in which persons living outside the area obtained treatment by the simple process of perverting the truth, but it was a matter in which we usually had the support of the inhabitants of the surveyed area who preferred not to have their 'pitch queered' by outsiders. At first it was necessary to treat all the patients (residents within the area) that attended, but later only those suffering from either fever or enlarged spleen were treated. In any case all patients who were obviously suffering from diseases other than kala-azar or malaria were advised, but told not to come again.

METHODS OF DIAGNOSIS

The methods of diagnosis we adopted were those which we have always employed in our out-patient department at the Calcutta School of Tropical Medicine and Dr C R Das Gupta who has been in charge of this treatment centre almost throughout the whole period had had a considerable preliminary training in these methods under the writer. The methods are roughly these — All cases showing a definitely positive aldehyde reaction were diagnosed kala-azar and treatment was commenced immediately. [Experience has shown that in Bengal, at least 98 per cent of these are kala-azar (Napier, 1922 and 1923)] The patients suffering from considerable splenic enlargement with a history of a long illness and usually with marked anæmia, but who had a negative or doubtful aldehyde test result, were diagnosed as 'splenomegaly, not kala-azar'.

and were advised to go elsewhere for treatment. All patients showing negative or doubtful aldehyde test results, who had fever (without obvious cause, such as pneumonia) and slight or no splenic enlargement, were looked upon as potential kala-azar or malaria cases, a blood film was taken, and they were put on a quinine mixture and kept under observation. Such patients usually attended regularly and little difficulty was experienced in coming to a diagnosis within a week or so, but in a few instances it was thought advisable to do a spleen puncture in order to save time. We never had any opposition to this operation, nor did we find that its performance detracted in any way from the popularity of the treatment centre. In this way we can confidently claim that a negligible number of false diagnoses were made and that, although in a number of instances there was a delay, practically no kala-azar patient who once presented himself escaped diagnosis eventually. During the second and third years of this investigation house to house visits were made and only a very small number of previously undiagnosed cases were brought to light.

The treatment centre was opened in April 1925. A qualified surveyor, who formed part of the staff of the inquiry during 1927-28, made a detailed map of the area, marking in each house or hut and giving it a number. At the same time he made a list of the residents, noting details of their religion, total number of persons, their age and sex, and the number of cases of kala-azar for each separate household. In the tables shown below all the figures (except those referring to kala-azar cases) show the state of affairs existent at the end of the year 1927, no correction for subsequent deaths and births has been made, but during the succeeding period the composition of the population has only undergone the normal change. By means of this detailed census we were able to check the statements of the patients regarding their place of residence. In a few instances it was found that the information given had been incorrect, in these cases, if the patient could not be traced to some other residence within the surveyed area, his case card was excluded from the analysis. From the end of 1927 a copy of the detailed census was kept in the dispensary so that the patients' statements could be checked and their actual residence noted immediately. We found that, although on the whole the information about patients was satisfactory, in a few villages in the northern part of the area, which were much nearer the road and less isolated than the rest of the villages in the area, cases were difficult to trace, that information regarding the number of residents, etc., was difficult to obtain and that all the kala-azar patients were not coming to us for treatment, we, therefore, decided to exclude these four villages from our analysis. Unfortunately most of the Mohammedans in the area lived in three of these villages, their exclusion detracted from the value of the inquiry, but in the interests of accuracy we felt it was essential that they should be excluded. The population of these four villages totalled 1,125 and up to the end of 1927 16 cases were traced to them.

In the girls' school referred to above there were about 40 boarders, and in the boys' school about 36. Most of these children went to their homes or

elsewhere during the holidays so that it was not possible to say where they became infected. Furthermore, the population of these schools was not a constant one, each year pupils left and others arrived. We, therefore, decided to exclude the pupils and teachers of these two schools from our analysis. Actually only 3 girls and no boys became infected during the period.

All the persons included in the analysis were permanent residents of the surveyed area, and, with the exceptions noted above, all the residents in the area were included in the analysis.

THE PHYSIOGRAPHY OF THE SURVEYED AREA

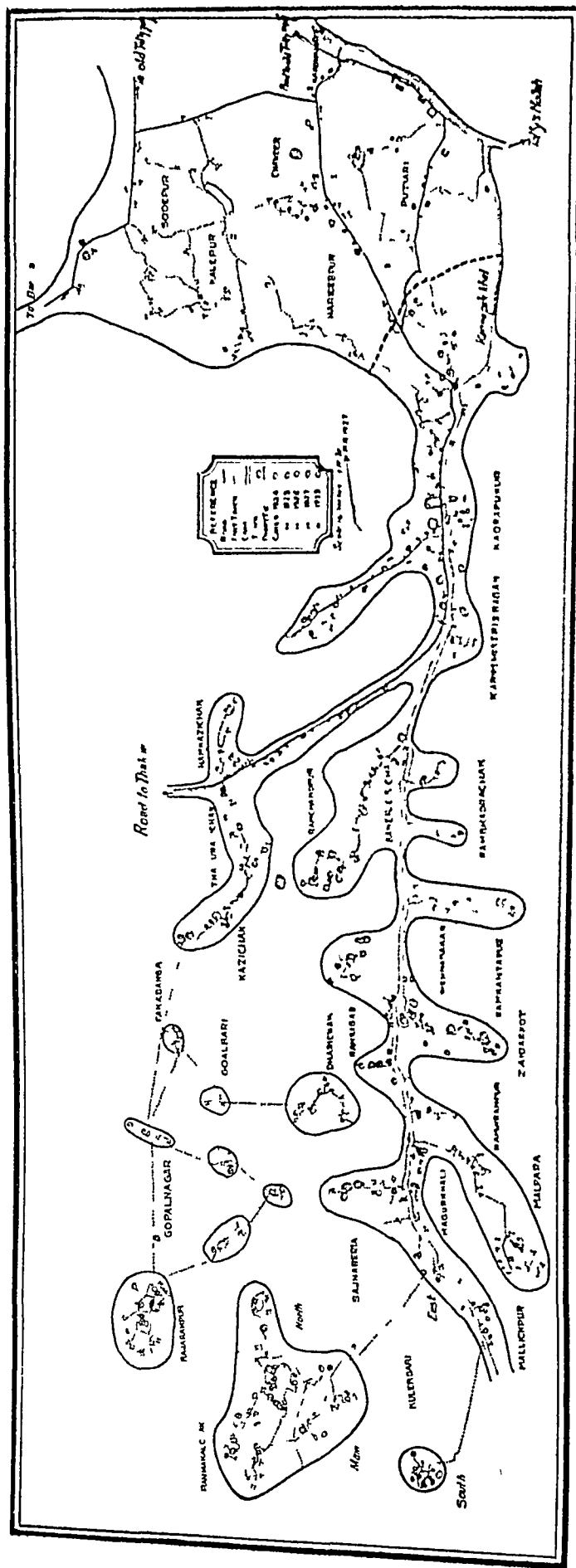
The area (after exclusion of the four villages) is about 7 square miles, $3\frac{1}{2}$ miles long and 2 broad. It includes 25 villages. A village consists of a number of huts grouped closely together on ground a foot or so higher than the surrounding rice fields. The huts are usually surrounded by thick vegetation, including a number of palms and other tall trees, which provide suitable shade. The intervening area consists of open rice fields which are flooded or partly flooded for about nine months in the year, that is from June to March. In some instances the rice fields entirely cut off a village from the adjacent ones, the only approach during part of the year being by boat along a narrow *nullah*. In others there is a narrow footpath which is dry all the year round, unless there are exceptionally heavy floods.

The area is about 20 feet above sea-level. The soil is alluvial. The subsoil-water level is very high throughout the year. The normal annual rainfall is 62 inches. The normal mean of daily mean temperatures throughout the year is 78°F , of the coldest months (December and January) 65°F , and of the hottest (May) 86°F . The normal annual mean of daily means of humidity is 77 per cent. There is a very low diurnal range of temperature for most of the year, and during the four months from July to October the diurnal range of the wet bulb is less than 3 degrees.

THE POPULATION

The population was 5,143 at the end of 1927 and has not undergone any abnormal change since. The inhabitants are all Bengalis, they are Hindus, 3,186, Mohammedans, 323, and Christians, 1,634. They live in 25 separate villages, the population of the villages varying from 642 to 26. In 9 villages, only Hindus live, in one, Hindus and Mohammedans only, in 2, members of all three communities, and in the remainder, Hindus and Christians. The inhabitants were classified according to their sex and whether they were adults or children, details of the members of each class are shown in Table I. All giving their age as more than 12 were classified as adults. They are all of the *ryot* class, most of them agricultural labourers working in the adjoining fields, but a few work in Calcutta, these as already stated, are almost all employed as mechanics by the Calcutta Tramway Company. Their economic condition is low on the whole but few actually suffer from malnutrition from this cause.

MAP SHOWING GENERAL LAY-OUT OF THE SURVEYED AREA



The original map from which this was reproduced was 6 feet long and in colour so that it is not to be expected that the reader will be able to see any of the details. The blank areas are rice fields. The *hal* which runs through the area from Tolly's Nullah to the north extremity of the map, is denoted by parallel dotted lines throughout most of its course (but in one place one of these lines has been omitted). The four villages north of the dotted line constitute the excluded area. There are a few unimportant inaccuracies in the map.

TABLE I

Showing population and kala-azar incidence in the surveyed area, according to community, sex and age

	MALES		FEMALES		BOTH SEXES		
	Adults	Children	Adults	Children	Adults	Children	TOTAL
HINDUS							
Population	973	626	1,050	537	2,023	11 63	3,186
Infected persons	45	53	39	49	84	102	186
Percentage infected	4 73	8 47	3 71	9 12	4 15	8 62	5 84
Population	1,599		1,587				
Infected persons	98		88				
Percentage infected	6 13		5 54				
MOHAMMEDANS							
Population	97	58	116	52	213	110	323
Infected persons	1	7	4	9	5	16	21
Percentage infected	1 03	12 07	3 45	17 31	2 63	14 55	6 50
Population	155		168				
Infected persons	8		13				
Percentage infected	5 16		7 74				
CHRISTIANS							
Population	475	315	476	338	951	683	1,634
Infected persons	44	56	33	49	77	105	182
Percentage infected	9 26	16 23	6 93	14 50	8 10	15 37	11 14
Population	820		814				
Infected persons	100		82				
Percentage infected	12 20		10 07				
ALL COMMUNITIES							
Population	1,545	1,029	1,642	927	3,187	1,956	
Infected persons	90	116	76	107	166	223	
Percentage infected	5 83	11 27	4 63	11 54	5 21	11 40	
Population	2,574		2,569				5,143
Infected persons	206		183				389*
Percentage infected	8 00		7 12				7 56

* The disparity between this total and that in other tables is due to the inclusion here of two patients from this area who were admitted into hospital under the writer late in 1924

A rough indication of the economic status of the various inhabitants is given by the composition of the households, the households of the better-to-do being on the whole larger, whereas quite a number of the households in which there are one or two persons are those of poor widows. There are, however, numerous exceptions to this generalization.

WATER-SUPPLY AND SANITATION

The inhabitants depend entirely on 'tanks' for their water-supply. There are a number of these in each village. Usually one or more tanks are selected as drinking-water tanks but these are seldom reserved for this purpose only, the inhabitants also wash themselves and their clothes therein. Recently, a tube-well has been sunk in Kaoriapukkur, but this is not popular as the people complain that the water from it is brackish.

An attempt was made to find out the source of drinking water of each household but this was abandoned as it was found that it was likely to vary from month to month. For any given tank it can be assumed that the households in which the water is used are those in its immediate vicinity. The *khal* is used only for washing purposes.

There is no system of sanitation whatsoever, the open fields are usually used for defæcation purposes.

DIET

Few of the people are actually indigent and most of them can afford two meals a day, yet a number suffer from malnutrition on account of the unsuitability of their diet. Their basic article of diet is, of course, rice. The Hindus supplement this by *dal* and vegetables and occasionally small fish, but the latter is somewhat of a luxury. The Christians and Mohammedans take in addition chickens and ducks, and also their eggs. Neither mutton nor beef is eaten. Milk is scarce and is not widely used.

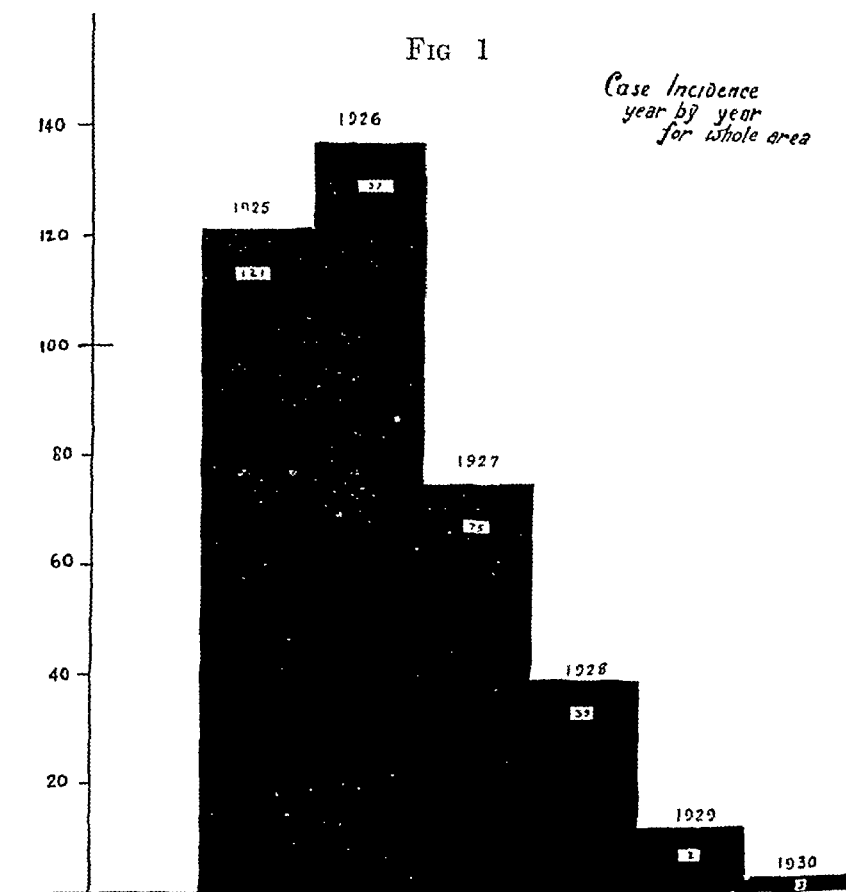
THE KALA-AZAR INCIDENCE

During the years prior to 1925 a few cases of kala-azar had been diagnosed and treated, but, though it was realized by the inhabitants that there was a considerable amount of this disease in the district, the very large majority of the sick were not making an attempt to attend any of the Calcutta dispensaries where suitable treatment was available. No rural treatment centre had been established anywhere near Kaoriapukkur. Their apathy was mainly due to ignorance but financial considerations also came in. During the first year the majority of the patients coming for treatment were advanced cases who had certainly become infected during the previous year or two years.

The Kaoriapukkur area is in a part of Bengal where kala-azar has been endemic for many decades. The disease is known to be subject to periodic waves of exacerbation. Taking Bengal as whole the peak of the present wave was probably at an earlier date than the commencement of the present

enquiry, but we were not able to ascertain that there had been any unusually high mortality amongst the population of this particular area during the previous few years, nor do the official fever mortality figures indicate this, and it was the general opinion in the district that the disease had only appeared in its present sub-epidemic form during the previous two years

Figure 1 shows the number of cases diagnosed each year. It does not indicate the incidence of the disease year by year as in many cases there was a considerable delay between the time of onset and the first attendance at our dispensary, this point will be discussed later in the paper. The actual

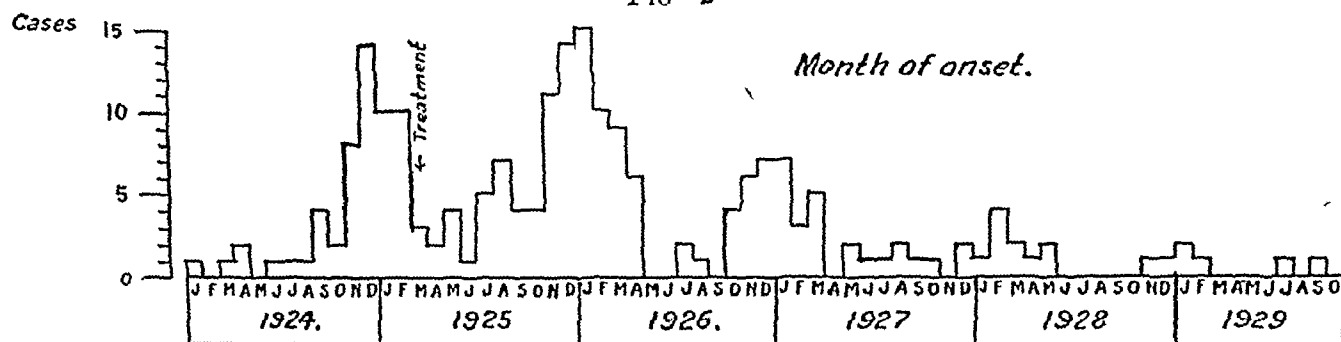


figures for the six years were as follows 1925—121, 1926—127, 1927—75, 1928—39, 1929—12, 1930—3, totalling 387 cases. The incidence was thus 75·2 per mille of the population (uncorrected, from our census during 1927) for the whole period, it varies from 26·6 during 1926 to 0·6 per mille in 1930. It is obvious that there has been a steady decline from the year 1926 onwards, culminating in an almost complete disappearance of the disease from the area by the end of 1930. The increase in incidence during the second year may be only apparent, as we shall show presently when the incidence village by village and community by community is discussed, the point, however, is an unimportant one.

SEASONAL INCIDENCE

The writer has already worked out the seasonal incidence in Bengal in an inquiry based on over two thousand cases. A rapid analysis of the case cards of this series shows that the seasonal distribution is very similar. The months of onset of the disease in 212 cases in the series in which this point was investigated more closely are shown in Figure 2. It will be seen that a sharp cold-weather rise occurs in each of the first three years and that even in the subsequent two years it was detectable.

FIG 2



INCIDENCE VILLAGE BY VILLAGE

The incidence village by village, year by year, is shown in Table II. In three small villages there were no cases, otherwise the incidence varies in each individual village from 2.72 to 23.65 per cent of the population.

INCIDENCE HOUSEHOLD BY HOUSEHOLD

There were altogether in the area 823 households. The number of persons living in a single household varied from 1 (in 18 instances) to 89 (in 1 instance). In most instances the single-person household consisted of a single-roomed hut in which a widow or a single man lived, it was usually situated near to, but quite distinct from, another group of huts which constituted the household of a family to which she or he was related.

The household in which there were 89 persons consisted of a large Hindu family of 4 generations. They lived in a closely grouped collection of huts, they were divided into sub-groups for purposes of sleeping but there was a good deal of common messing and it was not easy to subdivide them into definite separate households. In the average-sized households consisting of about 8 persons the food is cooked and eaten together, but there are usually two rooms in which the family sleeps. In the warmer weather the men and older boys sleep on the verandahs, the young children, girls and women inside. In the larger households, 15 to 20 persons, where two or more brothers live together, the wife of each family cooks the food for her family, but usually uses a common kitchen.

TABLE II

Showing population village by village, and kala-azar incidence village by village and year by year

	Village	Popu- lation	Cases coming under observation during							Percentage incidence
			1925	1926	1927	1928	1929	1930	TOTAL	
1	Kaorapukkur	246	7	4	1	0	0	0	12	4.88
2	Putiari	127	1	3	1	3	1	0	9	7.87
3	Karim mistri- bagan	147	2	1	1	0	0	0	4	2.72
4	Chakda	121	0	1	1	2	0	1	5	4.13
5	Dhalipara	399	17	10	3	1	0	0	31	7.77
6	Rambadrachak	63	1	2	0	0	0	0	3	4.76
7	Shamkazichak	102	0	0	0	0	0	0	0	
8	Zaiderkot	296	32	17	12	6	2	1	70	23.65
9	Ramchandrapore	254	18	23	3	1	0	0	45	17.72
10	Khasichak	167	4	18	4	0	2	0	28	16.77
11	Thakuranichak	265	8	12	4	2	0	0	26	9.81
12	Rajarampur	379	5	6	5	6	0	0	22	5.80
13	Ramnagar	212	17	10	6	1	1	0	35	16.51
14	Magurkhal	121	1	4	4	1	0	0	10	8.26
15	Krishnagar	110	0	1	1	0	2	0	4	3.64
16	Darirchak	152	0	1	2	0	1	1	5	3.29
17	Ramjanpur	56	0	2	0	0	0	0	2	3.58
18	Ramkantapur	54	0	0	0	0	0	0	0	
19	Goalbari	26	0	0	0	0	0	0	0	
20	Kulerdari	561	1	3	5	9	1	0	19	3.39
21	Rammakhal chak	642	2	9	10	2	1	0	24	3.74
22	Sajnabaria	325	3	6	4	0	0	0	13	4.00
23	Mullickpur	62	1	4	2	1	0	0	8	12.92
24	Malpara	104	0	0	5	2	0	0	7	6.73
25	Gopalnagar	152	1	0	1	2	1	0	5	3.29
	TOTAL	5,143	121	137	75	39	12	3	387	7.52
	Percentage	.	2.35	2.66	1.46	0.76	0.23	0.06		

If the household of 89 persons which cannot be strictly considered to be a single household is omitted from the calculations, the mean of the number of persons living in each household is almost exactly 6, actually the largest number of persons live in a household consisting of 6, but the figures for household of 5 and 7 are each almost equal to this whereas about two-thirds of the population of the whole area live in households consisting of 3 to 9 persons

Table III gives the distribution of the kala-azar cases amongst the households of different composition for the whole area together, and Table III *a, b, c, d,* and *e* the same figures separately for each of the 5 most heavily infected villages

Of the 822 households (excluding the household of 89 persons) 575 escaped infection altogether, 164 had one case only, 54 had two cases and in 29 there were three or more cases of the disease. That is to say, of the 387 patients 164 apparently had no other case of the disease in the household, 108 had another case of the disease in their households, either before or after they themselves became sick, and in 115 of the cases two or more other patients came from the same household

A few years ago the writer made certain observations regarding the epidemiology of the kala-azar in Calcutta. Amongst 100 houses in which kala-azar patients had contracted the disease he found that in 74 houses one case had occurred, in 16 two cases, and in 7, 2 and 1 three, four and five cases, respectively. He submitted these figures to Lieut-Colonel A. G. McKendrick who, after subjecting them to certain mathematical tests, concluded that they demonstrated a 'very marked degree of house infection'. If similar tests are applied to the figures which we have given in our Tables III, IIIa, etc., it will be found that in most of them there is an increase in the chance of the occurrence of another case in the house with the incidence of each successive case, or in other words that there is evidence of 'residual endemicity'. We have sent a copy of these tables to Colonel McKendrick and have asked for his opinion* in the meanwhile we have made no attempt to appraise mathematically the value of this evidence

On the other hand there is no evidence that an increase in the number of persons living in one household increases the chances of the members of that household contracting the disease, as is the case with most 'contagious' diseases. (This point should be considered entirely independently of the question of over-crowding which is considered elsewhere in this paper because, as a general rule, the increase in the number of residents is accompanied by a corresponding increase in the number of rooms.) If the households are divided arbitrarily into three groups as shown below (Table IV), it will be seen that the greatest relative incidence of the disease is in the small households there

* Colonel McKendrick has written a paper on the subject which will be found on p. 343 of this issue of the *Journal*

TABLE III
Showing the frequencies of the incidence of kala-azar cases in households of different numerical composition, for the whole area

Number of persons in household	Total Cases																							Total house-holds	Total Cases		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	31	89		
No Cases	15	33	81	88	90	71	59	32	23	16	10	10	4	5	2	2	4	3	1	1	2	2	1		575	0	
1 Case	3	12	20	23	30	19	16	10	8	6	5	4	5	0	2	0	1								164	164	
2 Cases		0	8	3	5	11	8	6	4	2	1	1	0	2	0	0	0	0	2	0	1				54	108	
3 Cases			1	1	2	2	3	2	1	2	1	1	0	0	0	0	1							1	19	57	
4 Cases				0	0	2	2	1	1																6	24	
5 Cases					0	1	0	0	0	0	0	0	0	0	0	0	0	0	1						2	10	
6 Cases						0	0	0	0	0	0	0	1												1	6	
9 Cases									0	0	0	1	0	0	0	0	0	0	1						2	18	
																									823	387	
																										Grand Totals	387
Total Cases	3	12	39	32	46	60	49	32	23	16	10	18	11	4	2	0	4	0	18	0	2	0	0	3	3	Totals	387
Total Persons	18	120	330	460	635	636	616	408	333	260	187	204	130	98	60	33	102	54	95	20	63	41	28	31	89	5 001	

is not much difference in the relative incidence in the last two groups, but the same tendency prevails

TABLE IIIa

Number of persons in household	Zaiderkot																Total Residences	Total Cases
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
No Cases	2	1	2	1	5	3	1	0	3	1							19	0
1 Case			1		3	3		1		1							9	9
2 Cases			2	1				2						1			6	12
3 Cases				1			1			1							3	9
4 Cases						1	2	1	1								5	20
5 Cases						1											1	5
6 Cases													1				1	6
9 Cases														1			1	9
																Grand Totals	45	70
Total Cases	0	0	5	5	3	12	11	9	4	4	6	11					Grand Totals 70	
Total Persons	2	2	15	12	40	48	28	32	36	30	13	38					296	

TABLE IIIb

Number of persons in household	KAZICHAK															Total Residences	Total Cases
	1	2	3	4	5	6	7	8	10	13	15	20					
No Cases	2	1	3	2	2	1	1		1	1	1					15	0
1 Case		2	1		2	1										7	7
2 Cases			1	1												2	4
3 Cases			1				1	1	1							4	12
5 Cases									.			1			.	1	5
													Grand Totals			29	28
Total Cases	0	2	6	3	0	2	4	3	3	0	0	5				Grand Totals 28	
Total Persons	2	6	18	16	10	18	21	8	20	13	15	20	.			167	

TABLE IIIc

Number of persons in household	DHALIPARA															Total Residences	Total Cases
	1	2	3	4	5	6	7	8	9	10	12	14	17	28			
No Cases	1	1	7	10	4	2	6	1	4	1		1	1	1		43	0
1 Case			3	4	2	2	2			1	1					15	15
2 Cases						2	1		2							5	10
3 Cases					1								1			2	6
													Grand Totals			63	31
Total Cases	0	0	3	4	5	6	4	0	4	1	1	0	3	0		Grand Totals 31	
Total Persons	1	8	39	56	35	36	63	8	54	20	12	14	34	28		399	

TABLE III*d*

Number of persons in household	RAMSAGAR																Total Recurrences	Total Cases
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
No Cases		2	5	4	1	2	1	1	0		0					0	18	0
1 Case			4	3		1	1	1	1						1		12	12
2 Cases			2		3	1	2	1			1						10	20
3 Cases					1												1	3
																	Grand Totals	35
Total Cases	0	0	8	3	9	1	5	1	1		2				1		Grand Totals	35
Total Persons	0	1	33	28	15	21	28	21	9		10				17			212

TABLE III*e*

Number of persons in household	RANCHANDRAPUR																Total Recurrences	Total Cases
	1	2	3	4	5	6	7	8	9	10	11	12						
No Cases	1	4	4	5	3	2	1	1									21	0
1 Case		1	4	2	3	5		1	2	1							19	19
2 Cases			2			1		1		1	1						6	12
3 Cases								1									1	3
4 Cases						1											1	1
9 Cases												1					1	9
																	Grand Totals	47*
Total Cases	0	1	8	2	3	11	0	6	2	3	11						Grand Totals	47
Total Persons	1	10	30	28	30	54	7	32	18	20	24							254

* This figure includes 2 cases treated by us in hospital during 1924

TABLE IV

Size of household	Kala-azar cases	Number of persons	Percentage incidence
Small households, 1 to 3 persons	54	478	11.30
Medium-sized households, 4 to 12 persons	236	3,739	6.31
Large households, 13 or more persons	47	846	5.55

Taking the instances in which there were two cases from one household, an attempt was made from the case cards to estimate the exact month of onset, the period which lapsed between the time of onset of the first and the second case in the household was then calculated. In three instances the time of onset in one or both cases was uncertain and these were excluded, of the remaining 51 the estimated period has been shown in diagrammatic form in Figure 3. Examination of this chart shows that certain distinct groups are

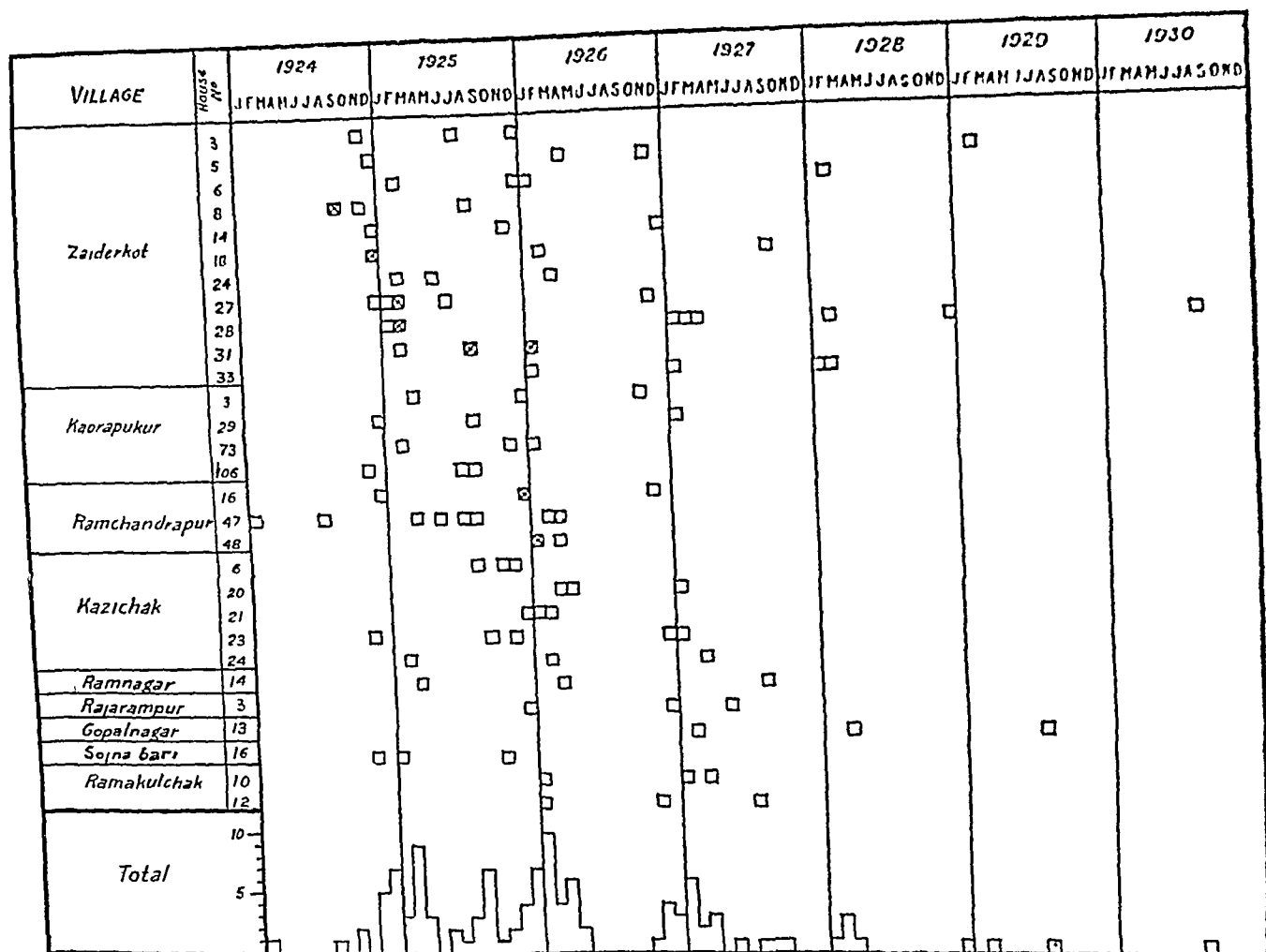
FIG 3

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

Intervals between the times of onset in households in which there were two cases

formed, firstly, there is a group at the beginning of the scale consisting of intervals up to 2 months, in these the infection was probably simultaneous. Secondly, there is a slightly larger—but less well-defined—group with its centre at about 9 months, the indication here is that the second patient acquired the infection from the first. This does not mean that the incubation period is 9 months because in very few instances did the patient come under treatment for the first three months and in most instances not until after the sixth month, that is to say the source of infection was present for an average period of six months. This observation would be in keeping with our independently-conceived opinion that the incubation period is usually from 4 to 6 months. Lastly, the remainder are spread out over a considerable period, in these instances there was probably no direct connection between the primary and the secondary cases. The apparent tendency to group at about six-month intervals may be connected with the periodic waves in the onset curve.

Fig 4



☐ Indicates one case
☒ Indicates two cases with onset in the same month.

INCIDENCE ACCORDING TO RELIGION

There are 9 villages with a purely Hindu population, of these the total population is 892 of which 51 persons were attacked, giving a percentage incidence of 5.72. Of these one village was very heavily infected, if this were excluded the figures for the remaining 8 villages would be population—725,

TABLE V

Showing kala-azar incidence year by year in different communities

		1925	1926	1927	1928	1929	1930	Total
	Numbers	41	77	41	17	8	2	186
Hindus, 3,186	Percentage incidence each year	1 29	2 42	1 29	0 53	0 25	0 06	5 84
	Percentage of total cases at each year	22 04	41 40	22 04	9 14	4 30	1 08	
	Numbers	12	4	3	1	1	0	21
Mohammedans, 323	Percentage incidence each year	3 72	1 24	0 93	0 31	0 31	0	6 50
	Percentage of total cases at each year	57 14	19 05	14 29	4 76	4 76		
	Numbers	68	56	31	21	3	1	180
Christians, 1,634	Percentage incidence each year	4 16	3 43	1 90	1 28	0 18	0 06	11 14
	Percentage of total cases at each year	37 78	31 11	17 22	11 67	1 67	0 55	

cases—23, and incidence—3 17 per cent. This incidence is lower than any but one of the 16 villages in which there is a mixed population.

The actual numbers and the percentages in the three communities of the persons attacked by the disease are given in the last column in Table V, the percentage incidence amongst Hindus is 5 84, amongst Mohammedans 6 50 and amongst Christians 11 14. Although the actual numbers of the persons attacked in the Hindu and Christian communities are about equal, the incidence of the disease in the populations of the two communities is in the latter case almost double that of the former.

This is one very definite fact that emerges from the inquiry.

In the case of the Mohammedans the numbers are very small, but the percentage incidence appears to approximate more closely to that of the Hindus than to that of the Christians.

There is some indication that the disease is not so prevalent in villages in which Hindus live alone, but the evidence is not very conclusive, as if the whole 9 villages in which Hindus only are living are considered the percentage incidence is almost as high as in the general Hindu community.

INCIDENCE ACCORDING TO SEX AND AGE

When the census was made the inhabitants of each residence were classed into four groups, male and female, adults and children, those whose age was given as 12 years or under were classed as children. The figures for these different groups with the kala-azar incidence in each group are given in Table I. In Table VI the age grouping of the infected persons in the surveyed

TABLE VI

Showing the age incidence in the two series, in the surveyed area and from other sources in India

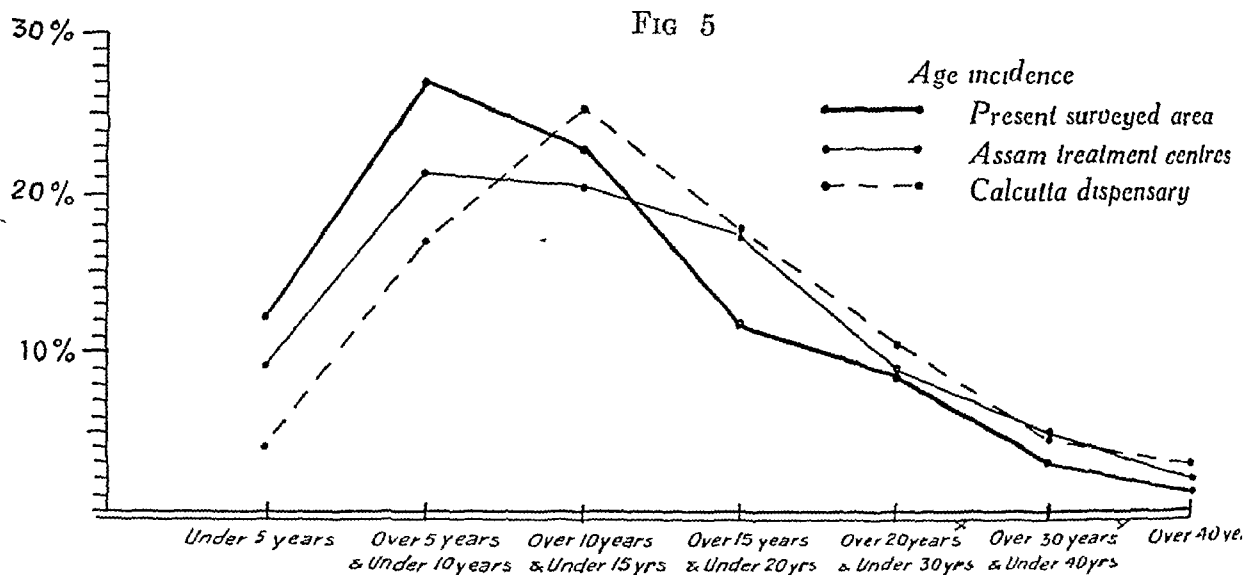
Age group	FIGURES FROM THIS SURVEY				FIGURES FROM OTHER SOURCES IN INDIA	
	Males	Females	Total Number	Percentage	Assam dispensary figures	Calcutta out-patient figures
					Percentages	Percentages
Under 5 years	22	26	48	12.10	9.3	11
5 years, but under 10 years	48	57	105	27.13	21.1	17
10 " " " 15 "	60	29	89	23.00	20.5	25.5
15 " " " 20 "	26	20	46	11.88	17.3	17.5
20 " " " 30 "	30	37	67	17.31	18.0	21.2
30 " " " 40 "	16	8	24	6.20	9.7	9.2
40 years or over	2	6	8	2.07	3.8	5.5
TOTAL	204	183	387			

area is given in more detail. For purposes of comparison the age distribution of the disease in India, according to figures from other sources, is also given.

The incidence amongst males is 80.0 per mille and amongst females 71.2 per mille.

The incidence amongst children, 114 per mille, is more than twice the incidence amongst adults, 52.1 per mille, this is probably a question of the greater susceptibility of the former class, but is one which will be taken up later. The mean age of the male group is 14.85 years and of the female group 14.62 the mean age of the male children is 8.70 and of the female children 7.88 years.

Comparison with figures from other sources—It is interesting to compare the age incidence curves drawn from the percentages given in Table VI. These are given in Figure 5. This shows that the nearer one gets to the source of the clinical material the younger are the patients. In the Calcutta dispensary figures (from our own out-patient department) the third quinquennial age group (10 to 15 years) is the largest. The majority of our patients come



* The fifth and sixth groups are 10-year periods as against 5-year periods of the first four, therefore the percentages were divided by two. The percentages in the last group were also divided by two.

from outside Calcutta, they are usually poor and, though money can be found to pay the fare of the bread-winner, the family cannot afford to send the children to Calcutta for treatment.

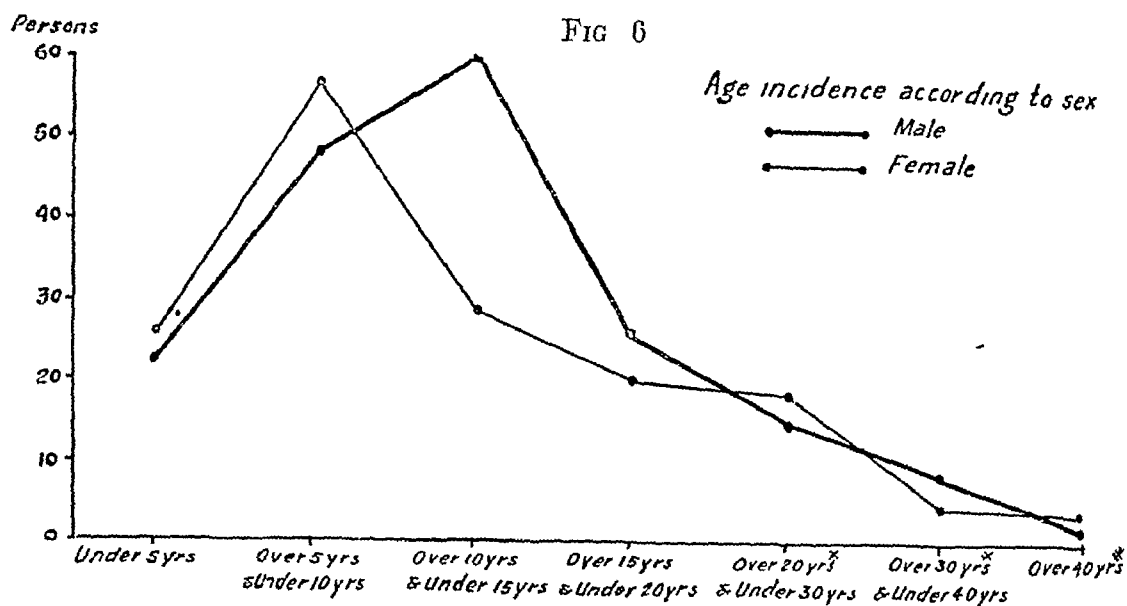
In Assam the dispensaries are widely distributed throughout the rural districts so that the majority of the patients have not long distances to come, but there are isolated villages with no dispensary near them. Thus, even here the dispensary returns do not indicate the true age incidence of them though they come much closer to it than the Calcutta figures do, the percentage of cases falling in the first quinquennial age group is twice as high in the Assam figures as it is in the Calcutta figures, and the percentages falling in the second and third quinquennial groups are about equal to one another.

In our surveyed area, where the dispensary was very near all the villages and where house-to-house inspection was carried out, the percentage of patients under 5 years is even higher and the largest percentage is in the second quinquennial age group, these figures probably indicate very accurately the true age incidence of the disease in India

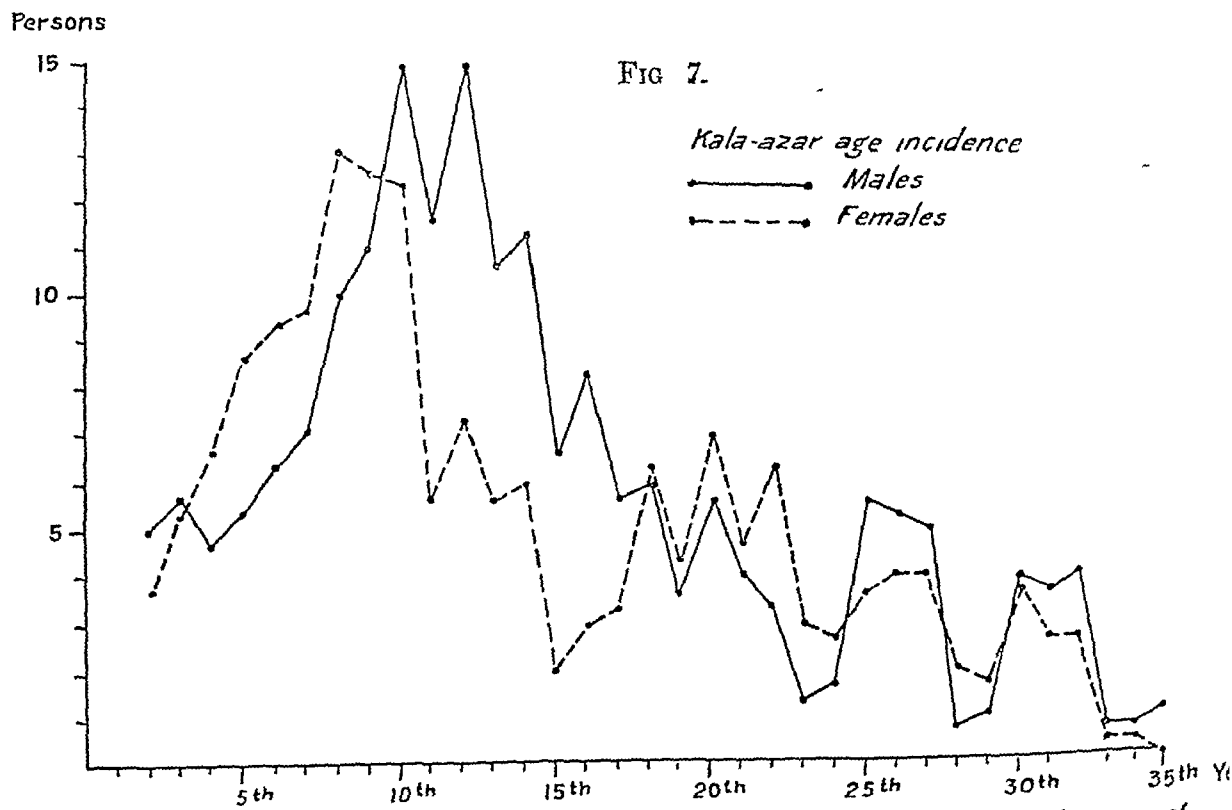
In the same way the apparent sex incidence of the disease is influenced by the distance of the dispensary from the source of the clinical material. In Calcutta (Napier, 1926) 76 per cent of our patients were males, in Assam 70 per cent were males, whereas in our present series the difference in the sex incidences has almost disappeared and only 53 per cent (52.96 per cent) are males

This difference in the number of infected persons in the two sexes is small compared with the figures from other sources in India, but is in our opinion too large to be attributed solely to errors of random sampling. Puidah is not observed to any extent in these rural districts and not at all by the Christians. A comparison of the figures for males and females in the two communities shows that $\frac{\text{Males}}{\text{Females}} = 1.11$ amongst the Hindus, and $\frac{\text{Males}}{\text{Females}} = 1.21$ amongst the Christians. The difference is insignificant and is the reverse of what we would expect if puidah were practised amongst the Hindus. We therefore separated the two sexes in the age distribution Table VI in order to see in which group the deficiency in the females occurred, and found that in the group, 11th to 15th year, there were only half as many females as males and that the deficiency in this group more than accounted for the deficiency in the whole female group. It was difficult to explain this deficiency on social or religious grounds and careful enquiries locally failed to elicit any reason which would account for the concealment of the disease in girls of these ages.

Here again a comparison between members of the two religions fails to show that the deficiency in this group is greater amongst Hindus. Had the deficiency occurred in the next age group, it might have been suspected to be due to shyness on the part of young married women. In actual fact the deficiency in the latter group was much less marked and was, we have every reason to believe, not due to this cause. We then plotted the age curve for each sex separately, and it was immediately apparent that the age distribution in the two sexes was different. Figure 6 suggests that there is a difference of about five years between the age of maximum incidence in the two sexes, but as this graph is based on five-year age groups a difference of a lesser period could not be shown. When we tabulated the frequencies in one-year periods it was found that, except in the case of the very young children, there were obvious mistakes due to patients giving the approximate age only, for example there were 9 girls aged 10, and 12 aged 12 years, whereas there was only one whose age was given as 11 years. The graph plotted from these figures was very irregular, and in order to obtain a smoother graph we took the average of the frequencies at each of three consecutive one-year periods, plotting it at



* The fifth and sixth groups are 10-year periods as against 5-year periods of the first four, therefore the figures were divided by two. The figures in the last group were also divided by two.



Showing the difference in the age incidence in the two sexes. At each year the mean of the yearly frequencies of a 3-year period is plotted.

the middle year period. This is shown in Figure 7. The male curve rises steadily up to the 7th year of life, then more rapidly up to the 10th year, this rise is maintained until the 12th year, there is a rapid fall until about the 20th year, after which the fall is continued but is slower, after this age period, however, the curve becomes very irregular on account of the smallness of the numbers and because patients persist in giving their ages approximately, choosing multiples of five. On the other hand the female curve starts to rise rapidly almost from the beginning, reaches its maximum at the 8th year, remains high for the next two years, then falls rapidly, it crosses the male curve between the 17th and 18th years and afterwards follows this curve more or less closely. That is to say for the first 17 years the female curve lags behind the male curve by about 2 years on an average. The comparative regularity of these two curves and the similarity of their general contour suggest that, except for slight inaccuracies in the ages given by the patients, our figures represent the true age and sex distribution in the area, and confirm our opinion that the deficiency in the females in the 11th to 15th year group is not due to concealment of the disease in girls of these ages.

Up to the age of 12 years there is practically no difference in the treatment of the children of the two sexes. A small percentage of the Hindu girls are married between the ages of 8 and 12, but even in these cases there is not much restriction to their movements. As the difference in the age distribution is mainly before this age, the difference cannot be a question of exposure to infection, it must, therefore, be a matter of the relative susceptibility of children of different ages in the two sexes. We felt that it would be interesting to see if the age incidence were the same in a heavily infected village. Taking Zaidarkot with its incidence of nearly a quarter of the whole population, we found that the incidence was 32.71 amongst children and 18.62 amongst adults, and that 11.43 per cent, 24.29 per cent, 24.29 per cent and 10.00 per cent of all patients in the village fell within 1st, 2nd, 3rd and 4th quinquennial periods respectively and that the remaining 30 per cent were 20 years or over. Comparison with the corresponding figures in Table I and Table VI will show that there is a very close similarity between the age distribution in this heavily infected village and that in the whole area but that the predominance amongst young children is less marked in the former. This is as we would expect where there is a very heavy infection persons of the less susceptible age groups are at greater risk of becoming infected, whereas the highly susceptibles are almost exhausted. With a 33 per cent incidence amongst all children the incidence in the most susceptible groups must have been very high indeed.

The subject of the difference in the susceptibility of persons of different ages to infection is a very wide one and cannot be entered into in any great detail in this paper. In this particular instance we think it is apparent that it is not a matter of 'salting' of the population. This would not account for the rise in the curve up to the 10th year and could hardly account for the extremely rapid drop thence to the 17th year. The sifting out of the

highly susceptibles and the production of immunity in the survivors by previous epidemics of the disease would account for the general decline in the age incidence curve after the 20-year mark but not for the earlier sudden drop which is shown in both curves in Figure 7. In our series the age recorded is the age at the time of first attendance. The majority of the patients, especially in the first two years (1925 and 1926) during which time the bulk of the patients attended, had been suffering for more than 6 months at the time they first attended, if in addition to this we take into consideration the incubation period of the disease, which probably, though not a constant period, is not less than four months, we are justified in assuming that the time at which infection occurred was about a year before the time of first attendance, so that if the curves are shifted one year to the left they would indicate the age at the time at which infection took place. In these circumstances the apparent extreme rarity of the disease in the first year of life is easily understood.

Let us consider the male curve, which rises at first slowly, then rapidly, reaching its maximum at the tenth year. This means that the most highly susceptible period commences during the ninth year, and that after the 11th year susceptibility declines rapidly. The changes in susceptibility from year to year are probably a matter of endocrine balance, and purely tentatively we put forward the suggestion that in this instance it is in some way associated with the development of the adrenal glands. These are well developed at birth, but rapidly diminish in size until the sixth month and then increase only slowly until the age of eight, after this development occurs rapidly until the age of 20 when the maximum is attained. The period up to the age of 8 years when, although general body growth is rapid, there is only a slight increase in the size of the gland amounts to a period of relative decrease. Thus, returning to our age-incidence curves (Figure 7), during the period of relative decrease in the adrenal gland there is an increase in the susceptibility to infection, then when the adrenals commence to develop rapidly there is at first no further increase and later a rapid fall in the susceptibility, after the 20th year when there is no further increase in the adrenals the kala-azar incidence stops its rapid descent and the subsequent slow decline is probably due to other causes.

We have no figures showing any difference in the age of development of the adrenals in the two sexes, but, as these are associated with the gonad development, which is much earlier in the female than in the male, in India at any rate, it seems possible that the development of this gland and of the balancing factor—whatever it may be—is much earlier in the female than in the male, this would account for both the earlier rise and earlier fall of the age-incidence curve in the female.

The difference in the total incidence of the disease in the two sexes can easily be explained by reference to the age-incidence curves. On account of the earlier rise and earlier fall in the female age-incidence curve, the period of higher susceptibility is shorter in the female than in the male, consequently

the difference in incidence between males and females up to the age of 17 represents the difference between the whole male and the whole female groups. This difference up to the end of each year of age (up to the age of 30, the later data being too irregular to be included) is shown graphically in Figure 8. There is a female predominance up to the 9th year, this is rapidly counter-balanced, and by the 17th year there is a marked male predominance, which is maintained at about the same level subsequently. It is thus probable that

Difference in
actual numbers

FIG 8

Kala-azar incidence in relation to sex

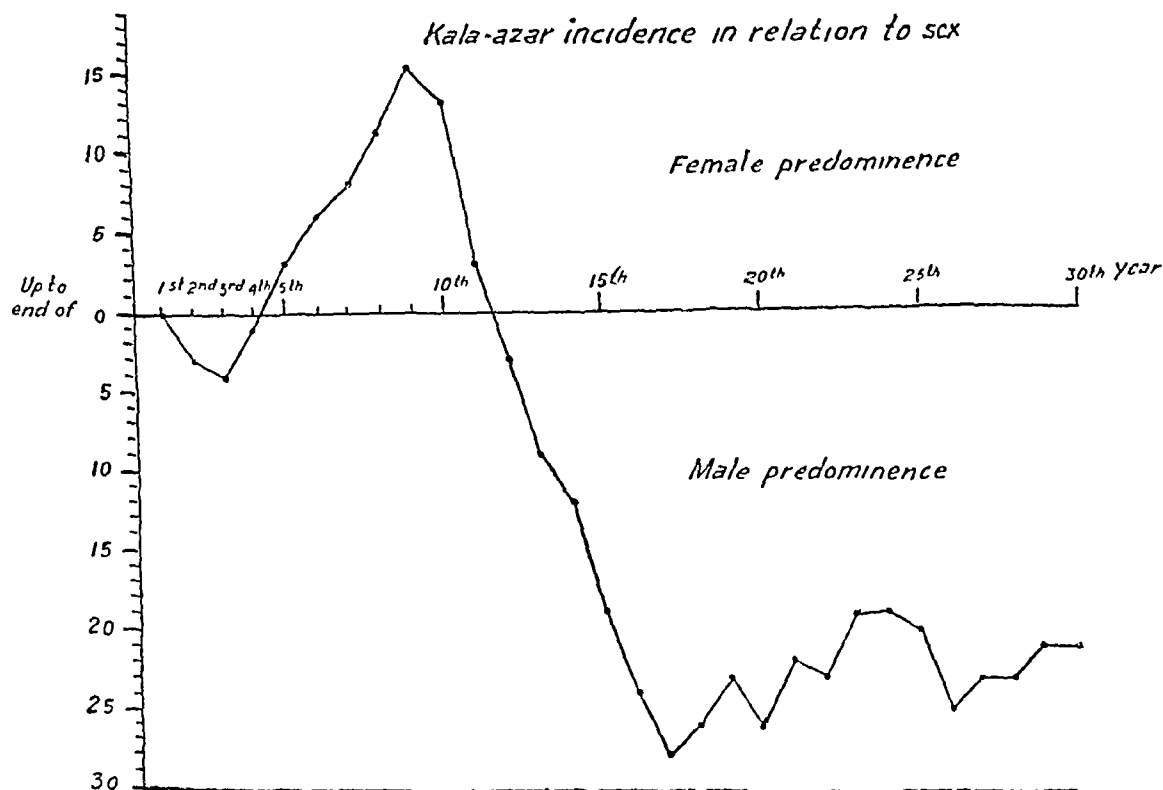


Chart showing male and female predominance up to various ages

this difference in the incidence in the two sexes is a real and not an apparent one

A CRITICAL EXAMINATION OF OUR FIGURES WITH REGARD TO THEIR ACCURACY

We are of the opinion that practically every case of kala-azar occurring within the surveyed area during the six years in which our treatment centre was in existence came under our notice and is included in this analysis, and that our figures give an accurate conception of the actual incidence of the

disease in the various villages, communities and age groups, and in the two sexes throughout the area

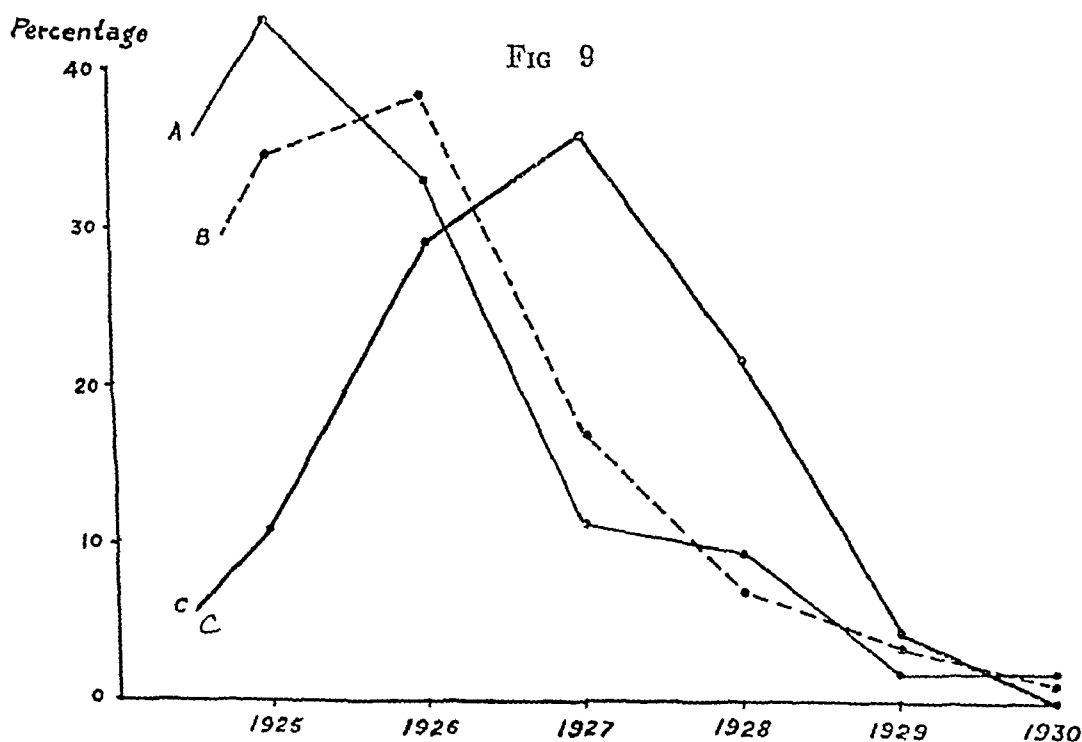
We will examine the figures which we have obtained to see what evidence we can obtain to controvert the above statement. We have already done this in the case of the age and sex distribution, and certain apparent irregularities have been explained. We will now take certain other factors which might adversely effect the correctness of our figures

Distance from the dispensary—The villages can be divided into three groups, those within a mile of the dispensary, those between one mile and two miles from the dispensary and those more than two miles from the dispensary. No village was more than three miles away. Table VII gives the incidence of kala-azar in each of these three groups. It will be seen that the incidence of the disease is not in proportion to the distance of the village from the dispensary, by far the largest numbers came from the second group, which was not only the largest group but happened to contain the two most heavily infected villages

TABLE VII
Showing the incidence year by year in different groups of villages

Group			1925	1926	1927	1928	1929	1930	Totals	Percentage incidence in each group
A	7 villages within 1 mile radius Pop 1,205	Numbers	28	21	7	6	1	1	64	5.31
		Percentage each year	43.75	32.81	10.94	9.38	1.56	1.56	100.00	
B	12 villages between 1 and 2 miles Pop 2,092	Numbers	85	94	41	17	8	2	247	11.81
		Percentage each year	34.41	38.06	16.60	6.88	3.24	0.81	100.00	
C	6 villages between 2 and 3 miles Pop 1,846	Numbers	8	22	27	16	3	0	76	4.12
		Percentage each year	10.53	28.95	35.53	21.06	3.95		100.00	
TOTALS			121	137	75	39	12	3	387	

Apparently the distance factor had little influence on the total number of patients eventually coming under observation. It will be seen, however, that the distance factor had a distinct effect on the time at which the infected persons came under observation. In Table VII the incidence year by year in each group is given and also the percentage of all patients from that group which the incidence in each year represents. A graph of these percentages has been drawn and is shown in Figure 9. The peak of the curve of Group A is in the first year, of Group B in the second year and of Group C in the third year, the same rapid decline occurs in each instance. That is to say, by the fourth year the disease had been practically eradicated from each of the three

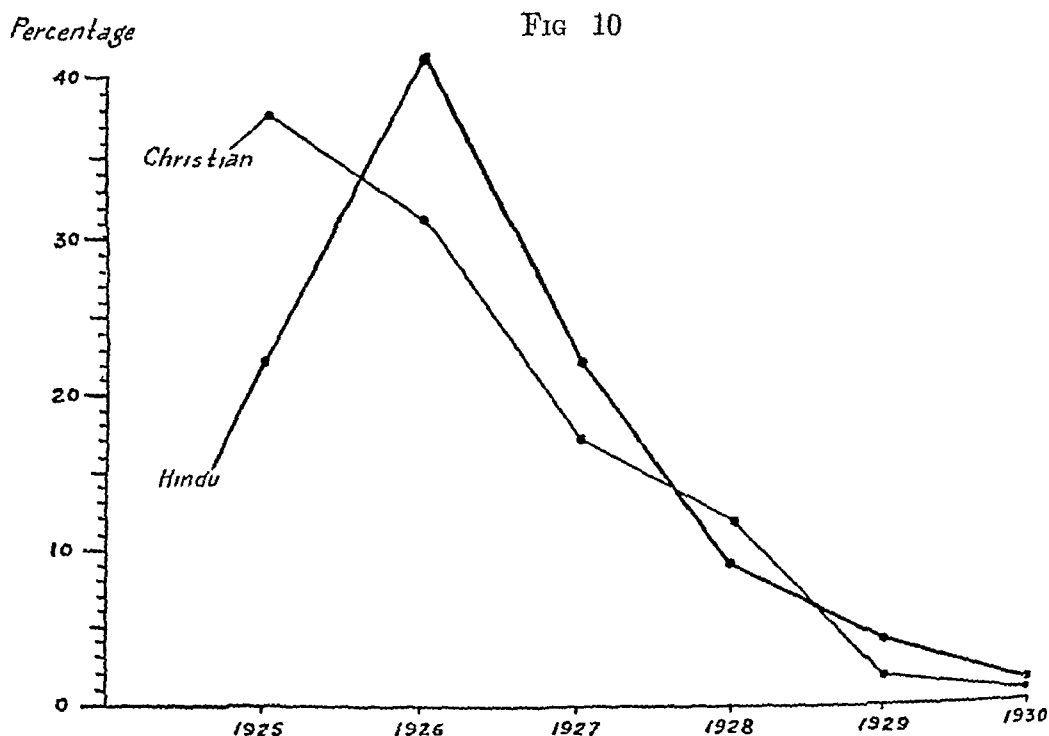


The incidence of kala-azar year by year in each of three groups of villages shown as percentages of total incidence in that group

groups of villages. Had, for example, a number of cases been overlooked in the villages of Group C, the disease would have continued to spread and in the later years, when all the villages were visited, a far greater number of infected persons would have been found in villages of this group than in the other villages where treatment had been more comprehensive. In actual fact the reverse was the case, only 3 infected persons were found in the 5th year and none in the last year. We are, therefore, justified in assuming that the difference in the relative incidence in the three groups of villages is real and not apparent.

The religion of the people—When the survey was commenced it was hoped that there would be a representative number of persons from each of the three communities, but circumstances, to which we have already referred, made it advisable to exclude certain villages from our calculation, these villages unfortunately included the bulk of the Mohammedans of the original area. The result is that this community is represented by such a small number that it is not fair to make comparisons between it and the other two communities which are each well represented.

The percentage incidence in the Christian community is very nearly double that of the Hindu. In Table V we have shown the number of patients attending each year, the percentage incidence of the disease each year, and the percentage of the total patients in that community that attended during each year, for each of the three communities. It is apparent from this table and from the graphs drawn from the percentages in this table (Figure 10) that there



Kala-azar incidence year by year in Hindu and Christian communities, respectively, shown as percentages of total incidence in each community

was a certain amount of hesitancy on the part of the Hindus in coming for treatment during the first year. At the same time we think that it is evident that during the second and subsequent years they came as readily as the Christians. The apex of the Christian curve is in the first year, that of the Hindu curve is in the second year, after this both curves show the same rapid decline, but it is not until the fifth year that the Christian incidence curve falls

below the Hindu incidence curve. Had there been any serious defection on the part of the Hindus this would have been shown by a much more marked lag in the fall of the Hindu incidence curve.

It might be argued that during the first year, the failure on the part of some of the Hindus to come for treatment would mean the loss of these cases, either through death or spontaneous cure.

This loss would be small and would be compensated for by the fresh infections in this community that would occur as a result of these persons remaining untreated for a longer period. The effect of treatment in stopping the epidemic extension of the disease is a point which will be discussed later.

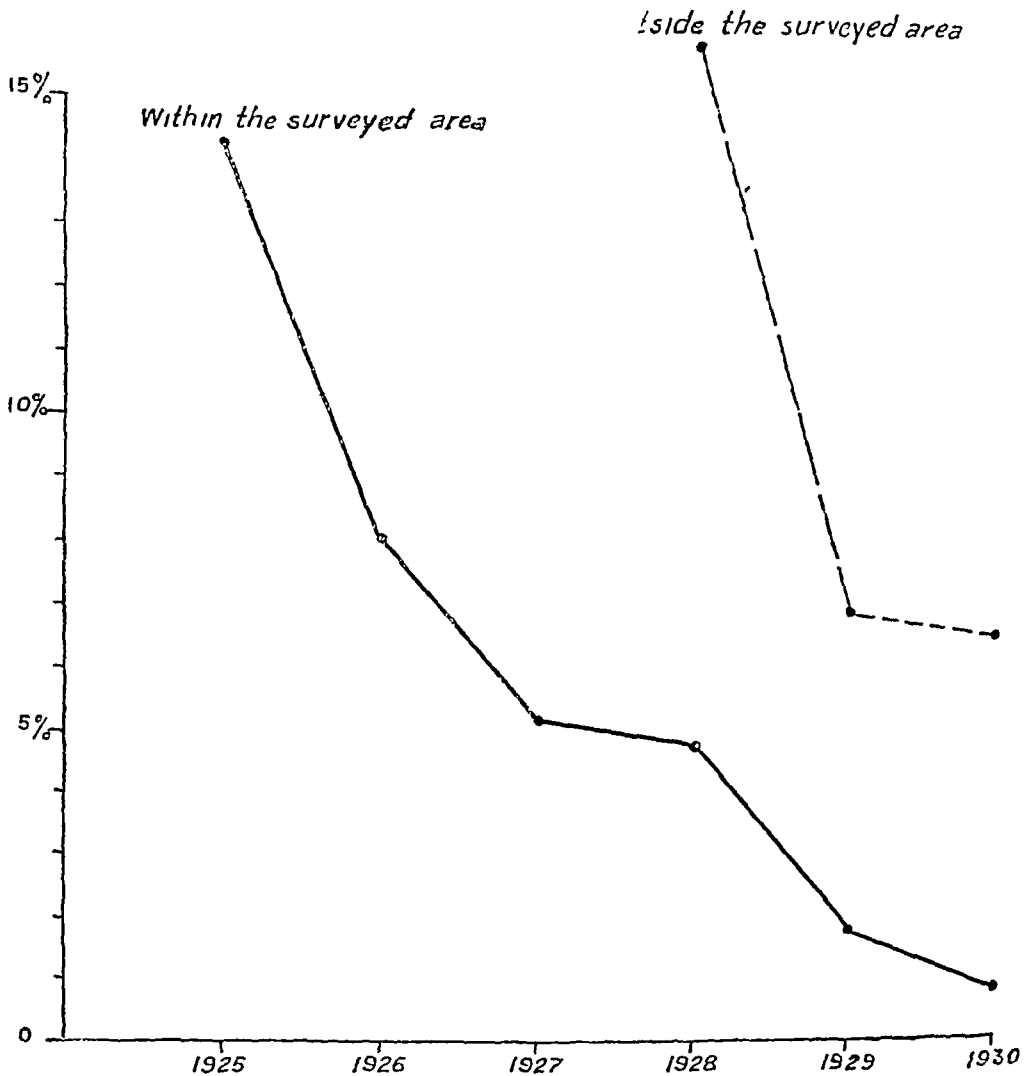
Thus, although the majority of the Hindus came under treatment at a slightly later date than did the Christians, we have every reason to suppose that the difference in the incidence of the disease in the Hindu and Christian communities is a real and not an apparent one.

THE EFFECT OF TREATMENT IN LIMITING THE EPIDEMIC EXTENSION OF THE DISEASE

One of the primary objects of this inquiry was to ascertain the effect of treatment of the affected persons on the epidemic extension of the disease in an endemic area. We, therefore, only treated patients who appeared to live within the prescribed area, our intention being at a later date to move our dispensary to an adjoining control area. Circumstances prevented us from carrying out our original plan, but from the beginning of 1928 we allowed patients from outside the area to come for treatment. Most of these persons attending came from just outside our area, but it was not possible to estimate what was the total population of the villages from which they came, nor was there any reason to suppose that they represented any considerable proportion of all persons suffering from kala-azar in these villages, as it is quite certain that some of the patients were going elsewhere for treatment.

The extremely rapid falling off in the incidence of the disease in the surveyed area, occurring as it has in a district where the disease is endemic, suggests very strongly that the influence of some unusual factor has been brought into action. The intensive treatment which was carried out in this area is, as far as we are able to tell, the only factor which is not common to the surveyed and the surrounding areas. As we have indicated above, it is practically impossible to estimate the percentage incidence of kala-azar in the surrounding villages for purposes of making a comparison with the known incidence in the surveyed area, the only means available for making this comparison is by comparing the percentages which the kala-azar cases constitute of the general attendance at the dispensary, separately for both the villages within and those without our surveyed area. These are shown in the following table (Table VIII) and have been graphically represented in Figure 11.

FIG 11



Kala-azar incidence year by year within and without the surveyed area, expressed as percentages of total dispensary attendance

TABLE VIII

A comparison of the kala-azar attendance with the general attendance from the surveyed area and from villages outside this area, year by year

Year	FROM VILLAGES WITHIN THE SURVEYED AREA			FROM VILLAGES OUTSIDE THE SURVEYED AREA		
	New cases	Kala azar cases	Percentage	New cases	Kala azar cases	Percentage
1925	856	122	14.25			
1926	1,782	143	8.03			
1927	1,599	82	5.12			
1928	924	44	4.76	462	73	15.80
1929	740	13	1.76	584	40	6.85
1930	497	3	0.60	262	17	6.49

By the year 1928 there had been a very marked reduction in the disease in the surveyed area, but in the surrounding area the incidence was still apparently very high, and in 1930 when the disease was practically eliminated from the former area, which had been subjected to six years of concentrated treatment, a number of cases were still cropping up in the latter area, where sporadic treatment had only been given during a period of three years.

Thus, the evidence in favour of the treatment of the kala-azar patients having limited the epidemic extension of the disease can be said to be fairly strong.

DERMAL LEISHMANIASIS

Of the 387 kala-azar patients from the surveyed area who underwent treatment six attended at some subsequent date suffering from post-kala-azar dermal leishmaniasis, that extremely interesting sequel of the visceral disease. This number constitutes 1.55 per cent for the kala-azar cases. The same condition was also found in 2 other patients who had never been treated for kala-azar but who gave a history of having suffered from fever a year or so previously.

A few weeks ago we sent a message to all the villages in the area asking all persons who had at any time during the last six years suffered from kala-azar to attend the dispensary at a certain hour. Considering that the patient could expect little immediate advantage from compliance with our request, the response was very good, altogether about 120 patients from the surveyed area mustered. They were examined hurriedly by the writer and Dr. Das Gupta. There was no evidence that any of them were still suffering from kala-azar, but six were found to be suffering from dermal leishmaniasis. Of these two were amongst the six in whom the condition had already been noted. In the other 4 cases the patients had not themselves observed that they had any skin disease. It is thus obvious that the disease is much more common than previous experience has indicated. Six cases out of 120 persons is 5 per cent, which may be taken as the probable percentage incidence of this sequela in a kala-azar population which has been subjected to the usual course of treatment by sodium antimony tartarate. Of the 10 persons with this condition that came under observation, the details of the treatment for the original visceral infection are available in 8 cases and show that these patients were all treated by sodium antimony tartrate and that the mean of the number of injections given was 27.25, the maximum being 30 and the minimum 19. They were all children under the age of 10 at the time the condition was first observed, the youngest being 5 years. Four were girls and eight were boys. The lesions were depigmentation only, except in the case of one girl who had commencing nodules on the face.

Discussion—This condition was not recognized at all until 1922 when Biahmachari described a nodular case. During the next few years a few more single nodular cases were encountered and described, by the writer

and by others, but the protean nature of the clinical manifestations was not fully recognized until a comprehensive study of the disease was made at the Calcutta School of Tropical Medicine (Acton and Napier, 1927, and Napier and Das Gupta, 1930). The attendance of new cases of this condition at the School of Tropical Medicine is now at least 100 cases a year. This gives a very inadequate idea of the number of cases of this condition which exist, as the patients seldom come unless the lesions are extensive. In our first series of 44 cases, 37 (or 84 per cent) showed some nodules, and in our second series of 150 cases, 110 (or 73 per cent) showed nodules, but in the present small series in only one out of 12 cases (or 8 per cent) is there any sign of nodular lesions.

The course of treatment which these patients received was not an inadequate one and from our experience recorded in the above-mentioned papers there is no evidence that the condition is more frequent after treatment by sodium antimony tartrate than after treatment by one of the pentavalent compounds of antimony, there is, therefore, no reason to suppose that these patients were open to greater risk of getting the condition than the rest of the kala-azar population. Though it is admittedly dangerous to generalize from the particular, there does not in this case seem to be any reason why we should not assume that at least 5 per cent of all treated cases of kala-azar in Bengal get this skin condition in some form or other.

The first signs of the condition appear usually about a year after the patient has been treated and cured—as far as all general clinical manifestations are concerned—of the visceral infection. The first lesions are either the erythema of the face or depigmented patches of the face or body. Very rarely the lesions progress until the extreme condition which simulates xanthoma tuberosum multiplex is produced. More frequently only nodules are produced, but in a number of instances the disease does not seem to advance beyond the depigmented stage. At one time we were under the impression that in all cases the nodular condition eventually developed, but the frequency with which only depigmented lesions are encountered in cases of comparatively long standing drives one to the conclusion that this is not the case. During the interval between the cessation of all symptoms of the visceral disease and the first manifestations of the dermal infection, which interval though usually only about a year may be a number of years, the parasites must be somewhere in the body and are most probably in the skin. If the parasite can remain in the skin for considerable periods without giving rise to any obvious lesions and if the lesions produced by the parasite in the skin may cease to progress at any stage, it seems quite possible that in a number of instances the parasite may be present and yet never produce any clinical lesions at all.

To carry the argument further, persons with very advanced lesions are seen but are rare, cases in which there are nodules are frequently encountered but form a very small percentage of the kala-azar population, cases in which there are only depigmented lesions are more common and form an appreciable

percentage of the whole kala-azar population, probably as high as 5 per cent, and finally the cases in which the parasites are present in the skin, but in which there are no clinical lesions, may be even more numerous and form a large percentage of all the treated kala-azar patients. These dermal infections whether accompanied by clinical lesions or not may constitute the reservoirs of infection.

OTHER DISEASES AMONGST THE INHABITANTS OF THE LOCALITY

Malaria—We have no accurate record of the spleen rate in the area, but as in all similar rural areas in Bengal, it is high. Malaria was the clinical diagnosis made at our dispensary in about half of the patients attending, and when pressure of work has allowed it this diagnosis has been confirmed by blood examination. In 770 slides examined during 21 months in 1926 and 1927 (September, November and December of 1927 being excluded), parasites were found in 492, the predominant parasite was *P. vivax* in 250, *P. falciparum* in 231, and *P. malariae* in 11. The highest incidence is in the months of October and November. There has not been any noticeable increase in the prevalence of this disease during recent years.

The frequency with which the history of the onset of the kala-azar attack simulates that of the typical attack of malaria, and the fact that the kala-azar onset curve reaches its height a few months after the period of highest malarial incidence, suggests that in this district a malarial attack is a common precursor of kala-azar.

Hookworm disease—This does not exist to any serious extent. Although a hookworm infestation has been noted in about 63 per cent of the population, it is usually a light one, in not more than 10 per cent of cases did the condition warrant treatment, and in only one case out of 143 examined was a severe hookworm anaemia noted. Dr A. K. Mukerjee, working under the direction of Dr A. C. Chandler at that time in charge of the Hookworm Research Department of the School of Tropical Medicine, who carried out some investigations in this area, was kind enough to supply to me the above information.

There has been no serious epidemic in the area since the influenza epidemic of 1918. Cholera and smallpox have occurred in mild epidemic form on two or three occasions during the six years. Typhoid has not been a recognizable clinical entity of any importance, although it is probable that a number of mild cases have occurred.

Respiratory diseases are common during the periods of weather transition, from hot to cold, or from cold to hot.

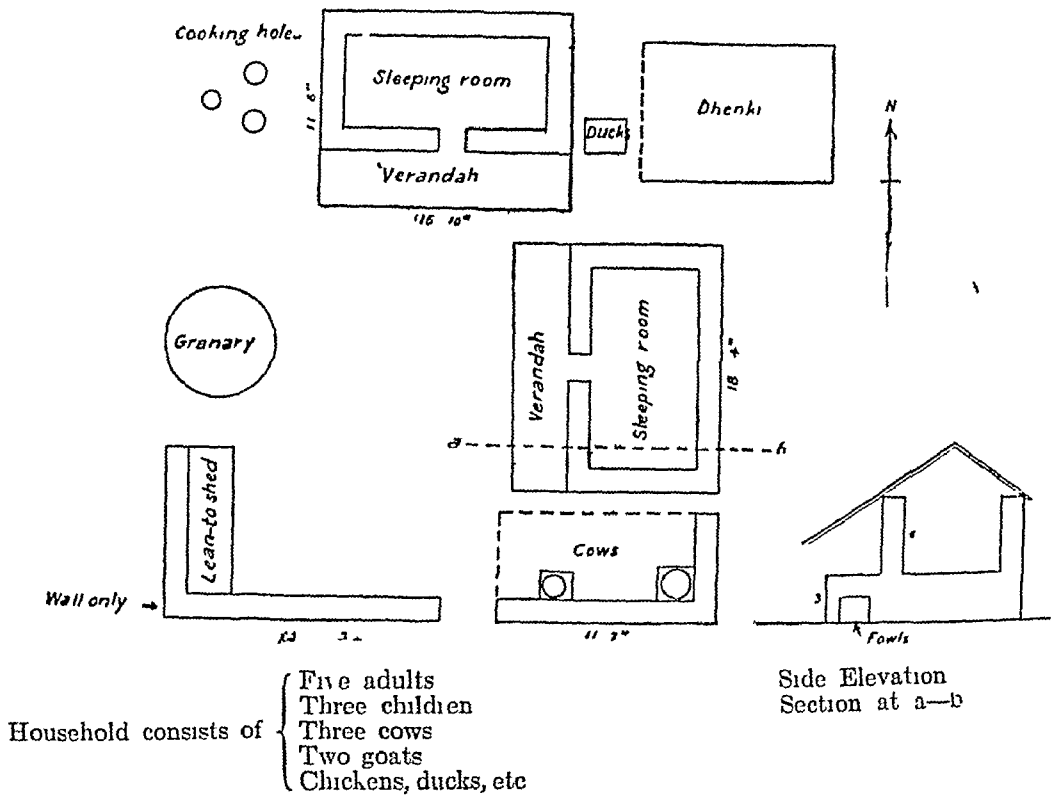
THE ENVIRONMENT ASSOCIATED WITH KALA-AZAR INCIDENCE

In the hope of obtaining information regarding any special environmental conditions which could be correlated with variations in the kala-azar incidence in different households within the endemic area, we made a detailed investigation of the conditions in each separate household in a number of the villages in the area. This investigation was carried out in 1927, the details given in

the tables are applicable to the state of affairs existent at that date, except with reference to the number of cases of the disease, this detail has been corrected up to the end of 1930

The nature of the building—Nearly all the buildings in the area are made with thick mud walls and thatch or *khola* roofing, a few have partial or complete galvanized iron roofing, but we have not differentiated buildings according to the nature of the roofing. Figure 12 shows the arrangement of a typical small group. The windows, if they exist, are usually completely closed in by bamboo matting, but there is frequently a small space between the roof and the walls through which smoke can escape. There are 10 groups of brick buildings, of

Fig 12
PLAN OF A TYPICAL HOMESTEAD



these two are infected. There are 86 persons living in these households, of which three have had kala-azan. The details are given in Table IX.

The kala-azan incidence amongst persons living in brick buildings is 3.49 per cent, against 7.8 per cent for the whole area in which this point has been investigated, the number of persons, however, is so small that the figure cannot be considered to be significant.

As we have stated above there are considerable differences in the sizes of the different groups. We have divided them into (a) single huts, (b) small groups, and (c) large groups. Details are given in Table IX.

The infection rate amongst persons living in single huts is distinctly higher than that for the whole area, 104 per mille, against 78. The difference between the incidence in the large and small groups is less and the incidence is, curiously enough, higher in the large groups.

Crowding and cleanliness of the surroundings—The average number of persons per room in infected huts is 3.94, as against 3.47 in uninfected huts, but, when the problem is examined from another point of view, there is a higher incidence in households in which there are less than four persons living in one room than in those in which there are more than four persons in a room. The two observations appear to be contradictory, but it is at any rate obvious that there is no evidence that crowding has any influence on the transmission of the disease.

It was noted whether the compounds or the areas surrounding the houses were tidy, or whether heaps of rubbish were allowed to accumulate. The distinction was necessarily a somewhat arbitrary one. The incidence was higher in the houses that were reported as untidy, but the difference cannot be considered to be significant.

The keeping of fowls and ducks—Full details are given in Table X. It will be seen that the incidence amongst the population and the infection rate amongst the huts each undergoes an increase of about 30 per cent above the mean where fowls are kept but that where ducks only are kept there is a decrease. The incidence is highest where both ducks and fowls are kept (110 per mille).

The incidence is also distinctly higher in households where the poultry are kept in the living rooms than in those in which they are kept elsewhere.

The keeping of cattle and goats—Full details are given in Table XI. Nothing very striking is brought out here. Where cows are kept a greater number of households are infected, but the incidence amongst the population is less. This is probably to be explained by the fact that the larger households usually keep cows, that each of these households consists of a larger number of persons, and that therefore the chances of each homestead becoming infected is greater than in the case of the small household.

Again, the incidence is slightly lower in the households in which cows are kept close to the sleeping quarters.

Where goats are kept the incidence is distinctly higher than the mean.

Discussion—On the whole this last part of the inquiry, that is, with reference to the environmental conditions, has been disappointing.

The higher incidence amongst the single-hut groups suggests that the disease is commoner amongst the poorer classes of the community.

The fact that the incidence is high, 110 per mille, in households where both fowls and ducks are kept and that high incidence is more definitely associated with the former is possibly a partial correlation with the fact that the disease is more common amongst Christians than amongst Hindus. On the other hand there is no doubt that the presence of fowls, especially when living in the

	Persons	Total Cases	170	11	45	40	286	369	106	142	103	86	150	226	123	52	97	121	70	346	161	2,716	7 18
			9	1	3	4	21	9	6	28	21	19	14	13	18	5	2	3	3	12	4	195	
Untidy	House holds	Total	12	19	11	13	18	36	18	21	27	13	21	19	15	10	9	12	9	33	20	336	32 14
		Infected	1	5	1	1	8	12	5	15	16	5	8	7	7	3	1	2	1	5	5	108	
	Persons	Total Cases	52	96	62	66	91	278	90	139	150	83	114	155	80	59	46	62	15	177	151	2,005	8 68
Pukka house	House holds	Total	4	2			1	1							1			1				10	29 00
		Infected		1			1															2	
	Persons	Total Cases	30	7			3	21							7			18				86	3 19
Mud hut	Infected huts	Persons Rooms	32 11	28 9	21 5	20 7	143 36	238 50	52 13	191 40	152 12	84 26	99 26	170 41	131 31	32 9	18 3	37 13	23 9	125 31	51 16	1,660 121	Persons per room 3 94
	Non infected huts	Persons Rooms	161 50	59 22	82 18	89 25	219 57	364 117	151 41	90 26	85 20	81 16	150 16	195 53	81 23	60 16	134 37	162 52	80 25	431 120	263 68	2,943 819	
	4 or more in a room	Persons Cases	82 5	36 3	86 3	45	213 21	318 12	122 7	182 47	104 20	97 15	117 13	102 7	151 22	49 2	103 3	64 2	11 1	295 5	189 5	2,161 193	Percent-age 7 88
Crowding or otherwise	Less than 4 in a room	Persons Cases	111 7	51 1	17 1	64 5	119 11	284 11	81 5	99 23	133 26	71 13	102 12	263 15	61 10	13 7	17	135 3	69 3	261 13	128 8	2,112 177	8 26

TABLE X
Keeping of fowls and ducks

	Percentage kala-azar incidence		41 89		10 46		33 84		7 48		29 15		6 07		32 86		9 09	
	Totals		191	80	1,339	140	464	157	3,342	250	943	100	2,388	145	70	23	185	15
Sajnabaria			13	2	114	4	30	6	233	9	21	4	141	5	4		22	
Kulderani			16	6	103	6	57	13	414	15	41	7	311	9				
Ramjibanpur and Rambadrachak			2	1	21	1	11	3	75	4	10	2	60	3	1		6	
Darirchak and Goal bari							26	4	153	4	26	4	155	4				
Krishnagar and Ramkantapur							21	3	114	3	21	3	114	3				
Magurkhal			1		6		12	4	82	7	11	4	76	7				
Ramnagar			21	13	121	21	26	18	161	27	9	6	59	8	4	1	19	2
Rajarampur			21	10	195	15	35	11	339	16	79	4	178	5	5	3	34	4
Thakuranichak			6	1	32	1	31	15	197	19	28	14	181	18	3	0	16	0
Khasichak							17	7	120	18	17	7	120	18				
Ramchandrapore			5	3	25	11	28	15	151	26	25	13	133	16	2	1	7	1
Zaiderkot			30	20	206	51	21	13	184	49	5	5	40	13	11	7	62	15
Mallickpore and Gopalnagar							26	6	152	8	26	6	152	8				
Rammakhalechak							59	15	530	19	59	15	530	19				
Dhalipara			33	15	246	19	30	14	232	21	10	4	51	7	13	5	65	5
Chakda							6	1	36	1	6	1	36	1				
Karimmistribagan			14	3	92	3	10	3	71	3					4		21	
Putiari			3	1	19	1	5	1	29	1	5	1	29	1	3	1	19	1
Kaorapukkur (Main)			26	5	159	7	10		67		4		22		20	5	114	7
Fowls kept	Total Infected	House holds	Persons		Total Infected		House-holds	Persons	Total Infected		House holds	Persons	Total Infected		House holds	Persons	Total Infected	
Ducks kept	Total Infected	House holds	Persons		Total Infected		House-holds	Persons	Total Infected		House holds	Persons	Total Infected		House holds	Persons	Total Infected	
Ducks but no fowls	Total Infected	House holds	Persons		Total Infected		House-holds	Persons	Total Infected		House holds	Persons	Total Infected		House holds	Persons	Total Infected	
Fowls but no ducks	Total Infected	House holds	Persons		Total Infected		House-holds	Persons	Total Infected		House holds	Persons	Total Infected		House holds	Persons	Total Infected	

No fowls or ducks	House-holds	Total Infected	10	14	4	18	18	15	11	8	19	12	14	4	6	10	5	8	28	11	224	29 40
	Persons	Total Cases	3	1	1	3	4	3	3	2	11	7	1	2	2	2	1		3	2	66	
Fowls, etc., living in hut	House-holds	Total Infected	41	59	15	79	80	124	44	35	95	49	51	13	32	29	33	34	116	60	1,018	8 15
	Persons	Total Cases	5	5	1	4	4	4	4	6	19	10	6	2	3	2	1		3	4	88	
Living under verandah	House-holds	Total Infected	2	2	1	1	6	10	16	4	12	9	17	9	15	3	7	5	12	9	159	17 11
	Persons	Total Cases	1	1	1	1	1	2	5	3	8	3	11	0	10	1	1	1	2	4	59	
Living in separate hut	House-holds	Total Infected	8	10	7	23	26	64	91	24	81	50	98	51	76	21	102	38	71	61	923	9 97
	Persons	Total Cases	1	1	1	1	6	2	5	12	17	5	14	0	15	2	2	1	3	5	92	
Living in adjacent hut	House-holds	Total Infected	21	4	7	2	27	35	1	28	10	8	13	20	11	1	3	16	30	12	257	36 96
	Persons	Total Cases	5	1	1	1	9	10	29	29	3	4	3	9	7	2	1	2	7	2	95	
Living in adjacent hut	House-holds	Total Infected	130	27	46	11	198	359	12	206	43	70	92	226	94	30	20	97	246	155	2,090	6 84
	Persons	Total Cases	7	1	1	1	12	14	47	47	3	13	4	14	10	3	1	2	7	1	143	
Living in adjacent hut	House-holds	Total Infected	5	1	1	1	7	1	4	3	4	1	3	12	3	4	2	1	5	7	62	33 87
	Persons	Total Cases	25	6	6	54	5	23	16	5	22	27	93	6	6	23	11	6	29	40	386	9 07
Living in adjacent hut	House-holds	Total Infected	2	3	5	2	3	9	6	10	10	1	1	1	1	1	2	1	11	3	72	33 33
	Persons	Total Cases	18	16	33	8	24	70	39	55	8	23	10	27	2	8	11	9	93	12	466	6 14

TABLE XI
Keeping of cows and goats

Percentage kala-azar incidence	33 47		7 39		27 84		8 85		32 86	
Total	481	161	3,435	254	273	76	1,310	116	90	92
Sajnabaria	32	6	228	9	16	2	87	1	13	1
Kulerdari	47	12	376	13	34	4	154	5	23	8
Rampbanpur and Rambadrachak	13	3	82	1	7		32		8	2
Darirchak and Goalbari	27	3	171	3	4	2	17	2	14	
Krishnagar and Ramkantapur	18	3	95	3	13		48		14	3
Magurkhal	12	4	82	7	6	2	29	2	6	2
Ramnagar	26	12	157	19	10	9	55	13	16	5
Rajarampur	23	7	243	11	21	9	143	11	11	2
Thakuranichak	31	12	192	15	17	7	72	10	15	5
Khasichak	11	6	106	14	15	8	63	14	9	3
Ramchandrapore	37	22	211	37	12	5	42	9	21	13
Zaiderkot	31	20	230	51	12	7	51	19	18	12
Mallickpore and Gopalnagar	20	7	123	10	17	2	73	2	9	3
Rammakhalehak	48	14	488	19	26	4	166	4	26	8
Dhalipara	34	15	218	22	27	8	129	10	18	9
Chakda	20	4	103	5	4		10		19	1
Karimmistribagan	15	1	93	4	3		14		12	3
Putiari	12	5	70	6	10	1	37	1	11	7
Kaorapukkur (Main)	21	2	135	2	19	6	87	10	14	2
	Total Infected	House-holds	Total Cases	Persons	Total Infected	House holds	Total Cases	Persons	Total Infected	House holds
	Cows kept				Cows not kept				Cows adjacent	

Persons	Total Cases	82	64	75	98	148	272	49	118	102	54	80	132	89	41	76	68	52	166	96	1,862	6 98
		2	6	3	5	14	11	3	26	16	5	7	3	6	4	3		2	9	5	130	
Cows separate	House- holds	7	1	3	1	16	23	11	13	17	5	16	9	10	6	1	13	5	21	19	203	31 18
		Total Infected		1		6	7	4	8	9	3	7	5	7	2		3	1	1	3	70	
	Persons	53	6	18	7	100	219	74	112	113	52	112	111	68	41	19	103	30	210	132	1,580	7 91
Goats kept	House- holds	4	2	1	7	5	15	19	14	14	1	5	15	10	10	9		5	23	19	178	41 57
		Total Infected	2	1		4	6	6	10	9	1	4	7	7	5	2		1	6	3	74	
	Persons	21	9	3	45	14	116	123	111	72	20	39	143	58	59	51		31	127	146	1,221	10 07
		2	2			7	8	9	33	12	5	5	9	10	8	2		1	6	4	123	

hut, adds very considerably to the production of general insanitary conditions, this would apply less in the case of ducks, as these birds tend to wander away from the house to adjacent water, they remain on the ground, and do not tend to come into the houses in the way that fowls do

The fact that the incidence is slightly lower in households where cows are kept may again be a partial correlation with the better economic status of the members of these households. The fact that fewer homesteads are infected in the class in which the higher incidence is noted rather adds to than detracts from any importance which one might attach to this observation. The fact that the incidence is lower where the cows are close to the sleeping quarters adds some slight weight in favour of our theory of zoophilism and sandflies, which has been propounded elsewhere. Goats add to the general insanitary condition and are probably a less suitable source of food than the cow.

KALA-AZAR INCIDENCE IN THE TWO BOARDING SCHOOLS IN THE AREA

In the early part of the paper mention was made of the two Christian schools in the area. The boarding scholars were not included in our general analysis for various reasons—the main one being that they are not permanently resident in the schools. In the girls' school 3 of the scholars have suffered from kala-azar, but none of the boys have contracted the disease. This is possibly a fact of some significance.

There are 36 boys in the school at present but there is accommodation for 52 and during the period of the enquiry the average number has been about 40. The school is situated in a comparatively isolated position on the edge of the village. It is separated by more than a hundred yards of open playing fields from other habitations and is surrounded on all sides by open playing fields and rice fields. There are very few trees and none of the thick undergrowth which is so commonly found surrounding the village homesteads. The boys sleep in a large open room, brick built, with a tile roof. It is well-ventilated, high and airy. A master sleeps in an adjoining room beyond which there is a cow-shed. There is a considerable amount of open space between the dormitory, and the fowl-house and kitchen, privies are used in both schools, they are clean but not protected from flies. The water supply is from a well in the compound, but tank water is sometimes used. The girls' school is in a less exposed position and is closer to the surrounding huts.

SUMMARY

Our observations were carried out during a period of sub-epidemic exacerbation of kala-azar in an area where the disease is endemic.

We have produced considerable evidence to show that during the 6 years a very large percentage of the cases of kala-azar came under notice and were diagnosed, and that our figures give the true distribution of the disease amongst persons of various ages and religions and of the two sexes.

In a total population of 5,143 persons 389 were diagnosed as kala-azar during the period of investigation. This is a total incidence of 75.64 per mille.

There was an apparent rise in the incidence during the second year but subsequently there was a steady fall to the present time when the disease has almost completely disappeared.

By making a comparison with the state of affairs in the adjacent areas considerable evidence has been produced that this decline in the incidence, if not brought about, has been at least considerably accelerated, by treatment of the infected persons.

By noting the interval between the time of onset in the primary and secondary cases in houses where more than one case occurred, it has been shown that in about one-third of these cases there was a possibility that simultaneous infection had occurred, but that in a big percentage of instances the intervening period was about a year.

The incidence amongst children (up to the end of the 12th year) is more than double that of the adults. We cannot visualize a mode of transmission which would render children more exposed to infection than the rest of the community, we must, therefore, assume that it is a matter of their greater susceptibility. The greatest degree of susceptibility appears to be exhibited in children from 8 to 10 years old.

The incidence is 80 per mille amongst men and 71 per mille amongst women, this small difference is probably real and is easily explained in the light of the observations regarding the age incidence in the two sexes. There is a very marked difference in the age of greatest incidence in the two sexes, there being an earlier rise and an earlier fall in the incidence curve by about 2 years in the female. This again is almost certainly a matter of earlier development of both susceptibility and of immunity in the female.

There is a considerable difference in the incidence of the disease in different villages, three small villages were actually entirely free from infection, in others the incidence varied from 2.72 per cent up to 23.65 per cent of the total population.

The percentage incidence amongst Christians is almost double that amongst Hindus. In eight out of the nine villages with a purely Hindu population the incidence is very considerably less than amongst the general Hindu population.

There is evidence to show that the leishmania infection persisted in the skin of a number of the persons treated for visceral infection, the figures indicate that 5 per cent (of treated patients) show clinical signs of the dermal infections and it is suggested that in a larger percentage the infection exists sub-clinically. The dermal condition was also seen in two other persons who had not had a diagnosed visceral infection and had received no treatment. It is put forward as an hypothesis that these persons constitute a source of infection, especially during hypo-endemic periods.

The population is heavily malaria-infected, and it seems possible that this infection plays an important part in the ætiology of kala-azar in the area.

When investigated household by household it was found that the greater the numerical strength of the household the greater were its chances of becoming infected, that the smaller the household the greater were the chances of each individual member of the household becoming attacked, and that there was some evidence of 'residual endemicity'.

An investigation of the living conditions in each household, especially with regard to domestic animals, did not bring forth any fact which could not be correlated partially with the observation regarding the greater incidence of the disease amongst Christians, or with the fact that the disease incidence is greater amongst persons of lower economic status.

The incidence of kala-azar in the two boarding schools at Kaorapukku was considerably below the average for the whole area, although the children were of the most susceptible age. The boys' school, in which no case occurred, is in a somewhat isolated situation surrounded by grass playing fields and open rice fields, not shut in by thick undergrowth as are most of the habitations. Cows are the only animals kept in close proximity to the sleeping quarters, and the buildings are of brick.

DISCUSSION

Three factors essential for the propagation of kala-azar are—the primary source of infection, the transmitting agency, and the susceptible population, the transmitting agency is influenced both by the local and by the climatic conditions. The absence of any one factor would mean that the disease would not spread. The story of the recent epidemic of the disease in some parts of Assam suggests that there both local and climatic conditions are always suitable for transmission and that the 'epidemic constitution' was brought about by a temporary increase in the suitability of the climate combined with a depression in the resistance of the population, possibly produced by the influenza epidemic, so that wherever infection was introduced in the form of a case of kala-azar epidemic conditions prevailed, some villages were infected early and were almost wiped out, some later, when the influence of the climatic factor was on the down grade and the population had recovered somewhat from the effects of the influenza epidemic and in these the condition was less serious, and a few escaped altogether through no cases of the disease being introduced. In the more highly infected villages the vicious circle was broken only by exhaustion of the susceptible population and in others by removal of the source of infection by treatment of the infected.

In endemic areas in Bengal, such as the one under investigation, when there is a wave of exacerbation of the disease it is more widespread and less catastrophic. Moreover it tends to last longer, unless the circle is broken by treatment of the infected persons. Few villages entirely escape infection but none are wiped out as the villages were in the severe Assam epidemics. There

is no history of the introduction of a case into a village causing an epidemic. Even in a family when one case occurs the secondary cases do not appear all at once, but are spread over a number of years, frequently occurring at intervals of a year. Under normal conditions children between the ages of 8 and 10 are mainly attacked, children of other ages less frequently, and adults comparatively rarely, and then possibly only when in a low state of health or when they have been rendered susceptible by some specific infection, such as malaria. In special circumstances when infection in a village becomes very intense the incidence amongst adults rises, but this does not occur frequently.

This suggests that the primary source of infection is always present, that the local conditions are suitable for transmission as also are the climatic conditions for some part of the year, and that the population always contains a few susceptibles. The balance is upset by a temporary increase in suitability of the climatic factor, or by the lowering of the general resistance of the population by some epidemic disease, and there is immediately a general exacerbation of the disease throughout the whole area. The variations in the degree of infection in different villages may be due to variations in the local conditions, or may be a matter of chance, in that the source of primary infection in the village happened to be very sparse at a time when the other factors in the 'epidemic constitution' were at their height.

The factor which limits the extension of the disease is obviously the individual resistance of the majority of the population against infection. Many are exposed to infection, few acquire the infection, and still fewer develop the clinical syndrome, kala-azar.

The fact that frequently in a household a single case of the disease crops up year after year led the writer to wonder how the source of infection was maintained in the absence of a clinical case of the disease and the question of an alternative host, mammalian, avian or reptilian, arose.

This point has been investigated by the writer as well as by other workers with entirely negative results, but our observations regarding the widespread occurrence of leishmanial skin lesions have made the alternative-host hypothesis unnecessary. These lesions are very chronic and would be a low-grade source of infection for many years. The relationship which these cases bear to cases of the visceral infection is parallel to that which carriers bear to the clinical types in certain bacterial diseases. It seems possible that this condition is evidence of an evolutionary move towards symbiosis, in which both the host and parasite population are playing a part, in the areas of well-established kala-azar endemicity.

If this theory is correct the incidence of skin infections should be a measure of the kala-azar endemicity in any particular area. In Bengal this incidence is high, from other areas in India few cases have so far been reported, but this is unfortunately not evidence that dermal leishmaniasis does not exist. We should not expect to find this condition in the upper Assam valley, but

we should expect to find more cases than have so far been reported in the endemic areas in southern India

The mode of transmission—One of the main objects of this inquiry was to throw light on the problem of the transmission of kala-azar. It cannot be claimed that the various observations regarding the epidemiology of the disease which we have made throw a spot-light on to the exact method by which transmission occurs, nor have they produced evidence definitely against any of the rival theories on the subject, but we can claim that all the observations can be fitted into the hypothesis that the sandfly *P. argentipes* is the transmitter and some of them add definite support to this hypothesis. On the other hand some of the observations also fit in equally well with the theory that transmission occurs by excretal contamination. The writer proposes not to expatiate at any great length on this subject, but to give summarily his interpretation of the observations.

The predominance amongst Christians might be due to the fact that persons of this class eat certain foods which the Hindus do not. On the other hand, the environment amongst which Christians live is much more suitable for sandflies than that amongst which Hindus live. The keeping of ducks and fowls in the compound, or even more so in the living room, is liable to foster a general insanitary state of affairs which would encourage flies and other insects likely to cause infection of food, at the same time such conditions, we have repeatedly shown, will encourage the breeding of sandflies. The same remark applies to the observation regarding the higher incidence amongst persons of a lower economic status.

The observation regarding the long interval between the times of onset in cases occurring in the same house and certain other observations seem to point, as we have already suggested, to the existence of a carrier state. If our conception of this state is the correct one it is difficult to see how infection could be transmitted except by the aid of a biting insect, whereas we have shown experimentally that the sandfly (*P. argentipes*) can become infected after feeding on a patient with the common form of dermal lesions, the depigmented patches.

The lower incidence amongst persons living in larger households might be used as an argument in favour of insect transmission, as it is an observation which has been made regarding malarial transmission, but it is against direct contagion and could scarcely be used in favour of the contaminative theory.

The absence of the disease from the boys' school is distinctly a point in favour of the sandfly theory. This school is situated in an open space, the room in which the boys sleep is high and airy, and some little way from any possible sandfly breeding ground. (Sandflies have not been found in the adjacent cow-shed.) No doubt great care is taken over the preparation of the food, but we see no reason why food prepared by servants and supplied to boys should be freer from contamination than that prepared by a housewife for her family.

The suggestions that treatment has checked the disease in the area and that the persons suffering from dermal lesions, who are frequently persons who

have received treatment, act as 'carriers' are not necessarily mutually exclusive, kala-azar patients are probably a much richer source of infection and their removal during a period when other factors were unfavourable would naturally check the epidemic extension of the disease

ACKNOWLEDGMENT

My thanks are in the first place due to my collaborator on this inquiry, Dr C R Das Gupta, M B, on whose shoulder more than a fair share of the clinical work fell. I felt that it was more convenient for one person to undertake the presentation of results, and that it was unfair to saddle Dr Das Gupta with any of the responsibility for some of the theories which have been evolved. My thanks are also due to Dr G N Sen, M B, D T M, who acted as additional clinical assistant for one year, Mr I B Bhattacharyya and Mr K M Dutta, who worked as surveyors at different times, and to John A Day, K N Dutta and B N Roy, insect collectors, all of whom have given valuable assistance in the inquiry.

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MATHEMATICAL ANALYSIS OF DR NAPIER'S STATISTICS
OF HOUSE INFECTION IN KALA-AZAR

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[Received for publication April 10, 1931]

THE figures relating to the whole area given in the accompanying table have been subjected to statistical analysis. In this table the various households are classified according to the number of their inhabitants, and also according to the number of those who contracted kala-azar. Thus in each compartment is entered a number $v(n, r)$, which denotes the number of households comprising n persons of whom r were infected.

As a first step it seemed advisable to determine roughly whether persons resident in large households were more, or less, liable to infection than those resident in small households. If one arbitrarily groups the houses in the sets 'less than seven,' and 'seven or more,' the figures are

	Number of persons in households of		
	Less than 7	7 or more	
Persons uninfected	2,017	2,659	4,676
" infected	192	195	387
	<hr/>	<hr/>	<hr/>
	2,209	2,854	5,063

whence $\chi^2 = 6.1$, and $P = 0.013$. That is to say if the chance of an individual becoming infected was independent of the size of the household of which he was a member, a result equally or more widely divergent would be expected to occur 13 times in 1,000 trials. Thus the correlation between infectedness and size of household is probably significant and it is to be noted

that the deviation is in the negative sense, that is to say the number of infections in large households is deficient, or in other words an individual who resides in a larger household would appear to be safer from the risks of infection than an individual who resides in a smaller one

Let us now examine whether a correlation exists between (a) the chance of a household becoming infected and (b) the number of its members. The figures are as follows —

	Number of households of		
	Less than 7	7 or more	
Uninfected households	398	177	575
Infected households	113	135	248
	<hr/> 511	<hr/> 312	<hr/> 823

whence $\chi^2 = 41.17$ and P is less than 1 in 10 million

A highly significant correlation thus exists, and in this case in the positive sense. In other words the larger the household the greater is the chance of an infection occurring within it.

The analysis may be further extended by considering how the chance of individuals who are members of an infected household varies with the size of the household. The figures are as follows —

	Number of persons in infected households whose members are		
	Less than 7	7 or more	
Persons uninfected	627	1,143	1,770
„ infected	192	195	387
	<hr/> 819	<hr/> 1,338	<hr/> 2,157

whence $\chi^2 = 27.35$, and P is of the order of 3 in 10 million. The correlation is highly significant, and is in this case in a negative sense.

So far then the figures suggest—

- (1) that the chance of a household becoming infected increases with the number of its inhabitants,
- (2) that the chance that an individual who lives in a larger household will contract infection is probably less than that of an individual who lives in a smaller one, and
- (3) that the chance of contracting infection in a household where infection has been present, is less in larger households than in smaller ones.

These conclusions are difficult to interpret, but are sufficiently definite to demand closer scrutiny.

If $f(n, x) dt$ denotes the chance that a household comprising n members, of whom x have been infected, will receive a further infection during the time dt then the variation of $v(n, x)$, i.e., $dv(n, x)$ will be

equal to the number of houses which are promoted from the class $(n, r-1)$, that is to $f(n, r-1)v(n, r-1)dt$ less the number, which by receiving a further infection are promoted out of the class (n, r) , i.e., $f(n, r)v(n, r)dt$. Thus we have

$$\frac{dv(n, r)}{dt} = f(n, r-1)v(n, r-1) - f(n, r)v(n, r) \quad (1)$$

For $r = 0$ this gives the solution

$$\log \frac{N_n}{v(n, 0)} = f(n, 0)t, \text{ (the log is to base } e), \quad (2)$$

whence $v(n, 0) = N_n e^{-f(n, 0)t}$, where N_n is the number of households with n inhabitants

By the usual methods of charting it appeared that an apparently linear relation existed between $\log \log \frac{N_n}{v(n, 0)}$ and $\log n$, of the form

$$\log \log \frac{N_n}{v(n, 0)} = a + \frac{1}{2} \log n, \text{ where } a \text{ is}$$

independent of n

$$\text{Thus } \log \frac{N_n}{v(n, 0)} = a \sqrt{n}$$

Comparing this with equation (2) it is clear that $f(n, 0)t = a\sqrt{n}$, where a is necessarily of the form kt , k being a constant independent of t , n and r . As however the observations refer to a particular time, so that t is a constant, we need only use the a values, and do not have to investigate the k and t factors separately. The value of the constant a was obtained by the method of least squares, and from it a was found to be 0.1574. From this it follows that

$$v(n, 0) = N_n e^{-0.1574 \sqrt{n}}$$

where N_n is given, and the only arbitrary constant is the number 0.1574

The calculated and observed values of $v(n, 0)$ are given in the first two rows of the adjoining table. In order to apply the χ^2 test to these rows, we must also take into account the calculated and observed values referring to infected houses, and calculate χ^2 from the 50 groups, and not from the 25 given in the table. It is a table of two rows and 25 columns, the totals of the columns being given, and one arbitrary constant being involved. As the calculated frequencies for n greater than 19 were small, the figures relating to houses with more than 19 occupants were grouped together, the number of columns being consequently reduced to 20. Thus the number of degrees of freedom is virtually $2(20) - 20 - 1 = 19$, also χ^2 on calculation is equal to 20.74, hence $P = 0.394$.

As the agreement between the actual and calculated numbers of uninfected households was so close, it was determined at this point to calculate the whole table of values $v(n, x)$ on the assumption that no other factors entered in

The general solution of equation (1) is known. If the cases of disease in the households all existed simultaneously it is clear that $f(n, x) = C_n (n-x)$ where C_n is independent of x , as, when there are x cases in the house, only the $n-x$ unaffected individuals will be liable to infection.

Thus $f(n, 0) = C_n n$

It has however already been shown that $f(n, 0)t = kt\sqrt{n}$,

whence $C_n = \frac{k}{\sqrt{n}}$, and with this value for $f(n, x)$ the solution of equation (1)

is $v(n, x) = N_n e^{-a\sqrt{n}} \frac{n(n-1)}{x!} \frac{(n-x+1)}{x!} (e^{a\sqrt{n}} - 1)x$, where, as before,

$a = kt$, and has the value 0.1574

The values of $v(n, x)$ as calculated by this formula, and the observed values are given in the adjoining table. The value of P for the numbers of houses in which one case had occurred ($x=1$) was 0.03 and for $x=2$, P was 0.01. Thus the fit becomes less satisfactory as x increases. It is to be noted that for $x=1$ the calculated figures are in general too great, whilst for $x=2$, $x=3$, etc., they tend to be much too small. There is consequently evidence that with each previous infection, the chance of the next infection is greater than that allowed for by the above theory.

The above calculations have been made on the assumption that all the cases of the disease observed, existed at the time of observation. It is possible, however, that the figures may refer to a period of years, and that some of the cases might have been removed by death or recovery before some or all of the others developed. Taking the extreme case where only one infected person was present in the house at any particular period, so that one could assume that, approximately, the number n of the inhabitants of the house is the number of persons liable to infection, the appropriate value of $f(n, x)$ will be independent of x and therefore equal to $k\sqrt{n}$. The solution will then

be given by $v(n, x) = N_n e^{-a\sqrt{n}} \frac{(a\sqrt{n})^x}{x!}$ where $a = kt = 0.1574$, as found

above. The values of $v(n, x)$ calculated from this formula deviate only slightly from the calculated values of $v(n, x)$ given in the table, and the following values of P are obtained. For the rows $x=0$, $P=0.394$ as before, for $x=1$, $P=0.05$, for $x=2$, $P=0.17$ and for $x=3$, $P=0.00001$. The agreement is of the same order when these two extreme assumptions are made.

It is clear therefore that under any intermediate circumstances the conclusions deduced from the above calculations will be valid

We have shown above that if we assume that $f(n, 0) = k\sqrt{n}$, the calculated frequencies agree closely with the observed. It is of interest to compare the results with those obtained if we assume that $f(n, 0)$ is proportional to n . The best value of k , as found by the method of least squares was 0.5887, the corresponding values of $v(n, 0)$ are as follows —

	Calculated	Observed		Calculated	Observed
$n = 1$	16.9	15	$n = 14$	3.1	5
$n = 2$	57.8	53	$n = 15$	1.6	2
$n = 3$	92.2	81	$n = 16$	0.8	2
$n = 4$	91.8	88	$n = 17$	2.2	4
$n = 5$	94.6	90	$n = 18$	1.0	3
$n = 6$	74.5	71	$n = 19$	1.6	1
$n = 7$	58.3	59	$n = 20$	0.3	1
$n = 8$	31.8	32	$n = 21$	0.8	2
$n = 9$	21.8	23	$n = 22$	0.5	2
$n = 10$	14.4	16	$n = 28$	0.2	1
$n = 11$	8.9	10	$n = 31$	0.2	0
$n = 12$	8.4	10	$n = 89$	0.01	0
$n = 13$	4.7	4			

whence $\chi^2 = 30.9$, and $P = 0.012$

An equal or greater discrepancy between the observed and the calculated results would be expected to occur only 12 times in 1,000

This new law is also unsatisfactory in as far as the deviations show a definite regularity, being positive for low values of n , gradually decreasing and becoming negative for values of n greater than 6. It appears however, that considerable deviations from the exact \sqrt{n} law would be consistent with the observed frequencies, but the most natural expectation, namely that $f(n, 0)$ was proportional to n , seems to be inconsistent with the observed figures

It is clear that whatever is the precise nature of the law there is evidence of intra house infection, because the deviations of the higher values of v shown in the accompanying table will be present on any assumption which excludes intra house infection. In fact, if for example in $n = 5$ or $n = 6$, we attempt to apply to the figures in the appropriate columns an equation of the type (1) when $f(n, x)$ is either independent of x , or of the form $C_n(n-x)$, the agreement will be unsatisfactory even although the best fitting values of the constants are taken and in the sense that the calculated results for the larger values of x are too low. This would seem to indicate that in the infected houses, intra house infection takes place. This would be consistent with the sandfly theory of the spread of the disease, but would also be true if transmission were by direct contact.

Discussion

It appears from the above that the chance of a household becoming infected increases with the number of individuals in it, and that the observed figures, when compared with those obtained on the assumption that the chance is proportional to the square root of the number of occupants, are of the magnitude to be expected as the result of random sampling and are therefore consistent with this view. Thus although it appears undesirable to insist on the exact validity of the square root law, it is of interest to enquire under what conditions results, at least approximately consistent with it, might be brought about.

First of all on the assumption that the disease is transmitted by direct contact, it is clear that there must be some limit to the possible number of contacts in a given time between uninfected and infected, in the sense that an infinite number of contacts would be impossible. Thus to take an extreme case, an individual residing in a household comprising a very large number of members, might reasonably spend most, if not all, of his time in intercourse with the other members of his own household, and so the household would be unlikely to become infected. Thus generally one would expect that the chance of the primary infection of a household comprising n inhabitants would be less than $k\sqrt{n}$, and the actual relation might approximate numerically to the square root formula, although in all probability it would not be algebraically identical with it.

A possible explanation of another nature suggests itself. Consider the analogous case of two spherical balloons of different radii each containing a gas A at the same pressure, and both suspended in an atmosphere of another gas B. Let the envelopes be permeable to the gas B but impermeable to the gas A. Then the chance that a molecule of gas B will enter a balloon is initially proportional to its surface area, and as the number of molecules in the balloons is proportional to their respective volumes, the chance of penetration by a molecule of gas B would be proportional to $n^{\frac{2}{3}}$ where n is the number of molecules contained.

Consider now the case of two tubular containers with impermeable ends. The surface area is in this case equal to the length of the cylinder multiplied by the perimeter of the cross section, and the number of molecules contained is proportional to the length multiplied by the cross sectional area. Hence in this case the chance of penetration is proportional to \sqrt{n} . Or to generalize, the chances of penetration of similar structures, impermeable at top and bottom, whose volumes are equal to length multiplied by cross section are proportional to the square root of the number of molecules which they contain.

The parallel picture is that of a number of one storey houses of equal height, but otherwise similar in shape, and of such an area that each inhabitant is allowed a definite amount of floorage, as is the custom in hospitals and like institutions. The walls are perforated by windows and doors, the number of

TABLE

	n-1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	28	31	89
Obs	15	53	81	88	90	71	59	32	23	16	10	10	4	5	2	2	4	3	1	1	2	2	1	0	0
Calc	15.2	52.0	83.7	84.0	89.3	72.1	58.0	32.6	23.1	15.9	10.1	9.8	5.7	3.9	2.1	1.1	3.1	1.5	2.5	0.5	1.5	1.0	0.4	0.4	0.2
Obs	3	12	20	23	30	19	16	10	8	6	5	4	5	0	2	0	1	0	0	0	0	0	0	0	0
Calc	2.6	12.2	23.9	27.5	32.9	28.7	24.9	14.9	11.2	8.1	5.4	5.5	3.3	2.3	1.3	0.7	2.1	1.0	1.8	0.4	1.1	0.7	0.4	0.4	0.3
Obs		0	8	3	5	11	8	6	4	2	1	1	0	2	0	0	0	0	2	0	1	0	0	0	0
Calc		0.7	2.3	3.4	4.7	4.8	4.6	3.0	2.4	1.9	1.3	1.4	0.9	0.6	0.4	0.2	0.6	0.3	0.6	0.1	0.4	0.3	0.1	0.2	0.3
Obs			1	1	2	2	3	2	1	2	1	1	0				1		0				1	1	1
Calc			0.1	0.1	0.3	0.4	0.5	0.3	0.3	0.3	0.2	0.2	0.1				0.1		0.1				0.04	0.1	0.1
Obs						2	2	1	1																
Calc						0.02	0.03	0.02	0.03																
Obs						1								1					1						
Calc						0.001								0					0						
Obs													1												
Calc													0												
Obs																			1						
Calc													0												
Obs																									
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these being proportional to the perimeter. If infection comes from without through the windows and doors, and in a random manner, then the chance that an infection will occur would be proportional to the perimeter, which in the conditions assumed, is proportional to the square root of the number of inhabitants.

It is evident that these conditions would be satisfied by the assumption of an insect vector moving at random in the neighbourhood, and entering through windows or other apertures in the walls. The necessary conditions are clearly many, and one would not imagine that they would be strictly fulfilled. The houses in the district from which the observations were collected might be similar in plan—we do not know. That the area of the plan of the house would vary directly with the number of its inhabitants would certainly not be true of European residences, though a closer approximation might possibly be expected in India. The number of windows might vary with the perimeter of the house, but probably not in a strict sense. Again it has been assumed that the members of a household contract infection within their own houses. If the sandfly bites only at night, as we have been told is the case, then this condition would generally be satisfied. In the absence of definite knowledge of local conditions it is impossible to say whether these assumptions are even approximately satisfied. All that can be said with definiteness, with regard to the \sqrt{n} relation is that it is not inconsistent with the assumption of transmission by the sandfly.

SUMMARY

It has been shown that the chance of a household becoming infected increases with the number of its inhabitants, and that the chance that an individual who resides in a larger household will contract infection, is probably less than that of an individual who resides in a smaller one.

Calculations have been made with the object of obtaining a quantitative relationship between the chance of infection of an individual living in an uninfected household, and the size of household in which he is living. The figures

agree well with the assumption that the chance is proportional to $\frac{1}{\sqrt{n}}$, so that the chance of an uninfected house becoming infected is proportional to the square root of the number of its inhabitants. Although too much emphasis should not be laid upon the exact accuracy of the \sqrt{n} relationship, the fact that it appears is very interesting, and should be further investigated. It appears to be consistent with the view that kala-azar is transmitted by the sandfly, although the hypothesis sketched out as to its possible origin will require criticism in the light of local knowledge.

The numbers of cases in which several infections occur in the same house appear to be too great to be explained without the assumption of intra house infection, an assumption which is consistent with the view of transmission by the sandfly as well as with that of transmission by direct contagion occurring within the household.

TRANSMISSION OF KALA-AZAR THROUGH *PHLEBOTOMUS ARGENTIPES* BY THE ORAL ROUTE

BY

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[Received for publication, June 22, 1931]

THE fact that *L. donovani* in its flagellate form has been proved capable of producing infection by the oral route necessarily suggested the advisability of investigating the possibilities of this method of transmission. So far as we know, the flagellate form normally only occurs in nature in the insect host *P. argentipes* and this led to the necessity of determining whether these insects were capable, by means of their contained flagellates, of producing infection by the oral route.

There are many ways in which such transmission might be effected, e.g., by infected flies dropping into water or food, being crushed by the hand in the act of biting, the contained flagellates being then accidentally transferred to the mouth, or by the flies feeding and being killed near the mouth. In any case the transference of flagellates to the mouth, however effected, would lead to the possibility of transmission of kala-azar by this means.

In order to test this hypothesis the following experiment was carried out —

Experiment

In order to obtain a large percentage of infected flies at a time of year when infections by natural feeding are lowest, i.e., in the winter months, numbers of *P. argentipes* were artificially fed, some with cultures of *L. donovani* and some with spleen and liver emulsions of hamsters or mice infected with kala-azar. The method of feeding is described in another account to be published later and need not here be specified.

The flies were only fed once artificially but were allowed to take their second and subsequent feeds, when these were taken, naturally on a rabbit.

The flies in all cases were kept as long as possible in order to obtain the heaviest infection. Each individual fly was dissected and all positive flies were used in the experiment. The infective material therefore was a mixture of flies, all infected, but some of which had had one feed only and the remainder of which had had a total of two to five feeds.

This material was ground up with a small quantity of citrated normal salt solution (Sod chlor 0.85 per cent, Sod cit 1.5 per cent) and administered *per os* to Chinese hamsters by means of a pipette which was not allowed to touch the hamster.

The details and results of the experiment are given below in tabular form —

Animal	Infective material	Total number of flies used	Number of administrations	Method of administration	Duration of experiment	Result
Hamster AT1	<i>P. argentipes</i> artificially fed on cultures of <i>L. donovani</i>	23	11	Oral	217 days	Negative
„ AT2	Do	15	7	Do	195 „	Do
„ AT3	<i>P. argentipes</i> artificially fed on liver and spleen emulsions containing <i>L. donovani</i>	39	21	Do	192 „	Positive
„ AT4	Do	46	15	Do	176 „	Negative
„ AT5	<i>P. argentipes</i> artificially fed on cultures of <i>L. donovani</i>	13	8	Do	178 „	Do

It should be noted that column 6 represents the time between the first administration of flies *per os* and the termination of the experiment by post-mortem of the animal. Actually an average period of over four months intervened between the last administration of flies and the date of post-mortem. The infected hamster showed no enlargement of the spleen and the infection was detected by culture of splenic material in NNN medium.

We do not think too much importance should be attached to the fact that the infection occurred in one of the hamsters fed on flies infected from emulsions as flagellation in *P. argentipes* is usually completed by the third day.

CONCLUSIONS

Out of two Chinese hamsters fed on *P. argentipes* artificially infected by feeding on emulsions of liver and spleen from kala-azar infected animals one became infected with kala-azar.

Out of three Chinese hamsters fed on *P. argentipes* artificially infected by feeding on cultures of *L. donovani* none became infected.

A NOTE ON THE EXPECTATION OF THE RELATIVE
PREVALENCE OF PLASMODIAL SPECIES WHEN
THIS IS BASED SOLELY ON THE RELATIVE
OUTPUT OF GAMETOCYTES

BY

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[Received for publication, April 16, 1931]

THERE has recently been published a most valuable *Memor*, Studies in the Parasitology of Malaria, *Indian Medical Research Memor* No 18, by Lieut -Colonel R Knowles, I M S , R Senior White and B M Das Gupta, the authors of which are to be congratulated on a valuable and comprehensive work But I should like to be allowed to criticize one small part—their calculation on pages 213—215 of the ‘ normal ’ prevalence of the three species of parasites—of what I term the ‘ expectation of relative prevalence, for owing to considering the relative gametocyte production rates for only the first cycle, I think that an erroneous conclusion was reached as to the expectation of relative prevalence, which in due course affected the judgment as to the prevalence of quartan malaria that was based on it

If we judge from the schizogony cycle alone and if further we assume that all individuals, sexual and asexual, are perfectly viable, then the scales are weighted far more heavily against *P malariae* than shown by the percentages calculated in the *Memor*—18 for *P malariae*, 39.3 for *P vivax* and 42.7 for *P falciparum* In order to prevent misconception I may say that this assumption of perfect viability is made only for the sake of argument to see the effect of the other factor alone As will be seen our final conclusion is that such viability is not only unlikely but is probably very different indeed for the three species

For any one species let x =the number of schizogony cycles,
“ “ “ y =the number of final asexual forms produced at
each segmentation,
“ “ “ z =the number of final sexual forms at each seg-
mentation

J, MR

Then we can calculate the number of asexual and sexual forms produced at each division as follows —

Cycles	Number of asexual forms after each division	Number of gametocytes produced by the last division
0	1	
1	y	z
2	y ²	y ^z
x	y ^x	y ^{x-1} z

So after x cycles the total number of gametocytes that would exist in the absence of the destruction of either sexual or asexual forms would be—

$$Z (1 + Y + Y^2 + Y^3 + \dots + Y^{x-1})$$

It is obvious that differences in the final value of this expression for the three species of parasites depends far more on the value of y and x than on the value of z. We shall more readily see this if we substitute the values that can be found from the figures given on page 214 of the *Memor*. For the period of time we will take n days and suppose n to be a multiple both of 2 and of 3 so that we are dealing only with complete cycles. Accepting the figures in the *Memor* for the average number of merozoites and the proportion of sexual forms, then

$$\text{For } P \text{ malariae } x = \frac{n}{3}, Y = \frac{16 \times 9}{26} = 5.54, Z = \frac{1 \times 9}{26} = 3.46$$

$$\text{For } P \text{ vivax } x = n, Y = \frac{25}{35} \times 17 = 12.14, Z = \frac{1}{35} \times 17 = 4.86$$

$$\text{For } P \text{ falciparum } x = n, Y = \frac{31}{41} \times 22 = 16.63, Z = \frac{1}{41} \times 22 = 5.37$$

As I need hardly say y has been calculated as the product of the average number of merozoites with the proportion of asexual forms to the total, and similarly Z for the sexual forms. Thus the total gametocytes at the end of n days will be as follows —

$$\text{For } P \text{ malariae } \quad 3.46 \left\{ 1 + 5.54 + (5.54)^2 + \dots + (5.54)^{\frac{n}{3}-1} \right\}$$

$$\text{For } P \text{ vivax } \quad 4.86 \left\{ 1 + 12.14 + (12.14)^2 + \dots + (12.14)^{\frac{n}{2}-1} \right\}$$

$$\text{For } P \text{ falciparum } \quad 5.37 \left\{ 1 + 16.63 + (16.63)^2 + \dots + (16.63)^{\frac{n}{1}-1} \right\}$$

If we wish to get a more easily realizable idea of the immense differences in the values of the first expression as compared with the other two, let us consider only the largest terms in each—the last, and let us take a definite period

of say 24 days from the first sporozoite infection as representing a low average period of infection in man

Then the values of the last terms will be as follows —

For <i>P. malariae</i>	$(5.5)^7 \times 3.5$
For <i>P. vivax</i>	$(12.1)^{11} \times 4.9$
For <i>P. falciparum</i>	$(16.6)^{11} \times 5.4$

For those who are not satisfied with this general picture I have worked out the ratios of the approximate values of the complete expressions for the total number of gametocytes after 24 days under the conditions assumed

Ratios of number of gametocytes —

<i>P. malariae</i>	1	} or 1/66 millions
<i>P. vivax</i>	66 millions	
<i>P. falciparum</i>	2273 millions	

These and similar figures for other periods are of *no particular use* in further calculations, for it must be remembered that such figures only give us the relative chances of propagation of the three species *in the first round*. Thus if we suppose that the numbers of gametocytes produced in a given period of n days by the three species all starting level are in the ratio of 1 : 100 : 1,000 for *P. malariae*, *P. vivax* and *P. falciparum* and if we suppose that the number of infections produced after the required interval, say m days, by mosquitoes at the end of the first round will be in this same ratio of 1 : 100 : 1,000, then in the next round, since the same factors will operate in each case the same way as in the first n days, after another period of n days ($n+m+n$ days after the start) the total gametocytes will be in the ratio of $(1)^2$: $(100)^2$: $(1,000)^2$, i.e., 1 : 10,000 : 1,000,000, which represents the ratio of the chances of the propagation of the three species in the *second round*.

Further calculation is not needed for us to see that *if the only factors* affecting the relative prevalence of the three kinds of malaria were differences in the time of the schizogony cycle of each species, and differences in the gametocyte output, then *P. malariae* has only the shadow of a chance and *P. vivax* only a small chance of propagation compared with *P. falciparum*, and that the chief reason for this is the larger value for x , the number of segmentations in a given period, in the case of the last two and the large value for y , the *asexual* form output at each segmentation, in the case of *P. falciparum*. It is these, and not the proportion of sexual forms at each segmentation, that have the most force in determining the final great difference in gametocytes.

In the *Memoir* the first cycle alone was considered and the conclusion was reached that the prevalence of the quartan form was generally *less* than what one would expect from a consideration of the effect of these two factors only. I think I have sufficiently demonstrated that if more cycles and more man mosquito

'rounds' be considered the *reverse* is the case--that the prevalence of quartan is inconceivably greater than one can expect from these two factors *alone*

The odds against *P. malariae* are weighted so heavily, that, seeing that these odds do not correspond to the facts of prevalence, *we must conclude that other factors are at work*. Chief of these I should imagine would be *differences in the viability* of the asexual forms of the three species after each segmentation, because of differences in *their immunity* to man's defensive reactions. It looks as if there is something very different in the general make up of the quartan parasites compared with the other two species. The difficulty of curing quartan malaria points in the same direction. However this is not my subject. What the other factors concerned may be I leave to malariologists. My excuse for this note is that it demonstrates that *in addition* to the factor of great differences in gametocytes production *there must be some other more important factor or factors to explain the facts of species prevalence*.

A SUMMARY OF THE RAT-FLEA SURVEY OF THE MADRAS PRESIDENCY WITH A DISCUSSION ON THE ASSOCIATION OF FLEA SPECIES WITH CLIMATE AND WITH PLAGUE

BY

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[Received for publication, April 30, 1931]

Introduction.

THE Indian Research Fund Association kindly financed this survey of the rat-fleas of the Madras Presidency. The survey started in September 1928 and ended in April 1930. It has been conducted by one or the other of us two at different times with the assistance of the following officers who were responsible for the field work —

- (1) P V George, B A, L M S, B S Sc, who has worked throughout the whole inquiry
- (2) N Natarajan, M B, B S, B S Sc
- (3) D S Mankikar, M B, B S
- (4) Jemedar F Jesudasan, I M D
- (5) P V Seetharama Iyer, M A, Zoologist at the King Institute, Gundy

Thirteen reports(1) embodying about thirty local surveys have been published as mentioned in the reference list which should be consulted when individual surveys are referred to. Maps 2 and 13 show the different areas that were surveyed. Most are towns, but some include not only the chief towns from which the area is named but also neighbouring villages and smaller towns. These areas were chosen

to be as representative as possible of the Presidency. The only part of the Presidency not surveyed is the Vizagapatam Hill Tract, which was not touched for lack of time, but, since it is essentially different in many ways from the rest of the Presidency, its non-inclusion does not affect the summary. The large Indian State of Mysore was not surveyed, but care was taken to include a few places that, though they were politically not in Mysore, yet formed part of the same plateau area. Later on we intend to survey a few places in Mysore, Travancore, Coorg and the Vizagapatam Hill Tract and so *complete* a survey of South India.

The exact methods used, details of baits, etc., are given in the individual reports particularly in the first. Each local survey lasted about three or four weeks.

For the sake of better understanding this summary, we shall begin by a brief description of the geography of South India and then go on to give details of our results and of the conclusions drawn therefrom.

Geography.

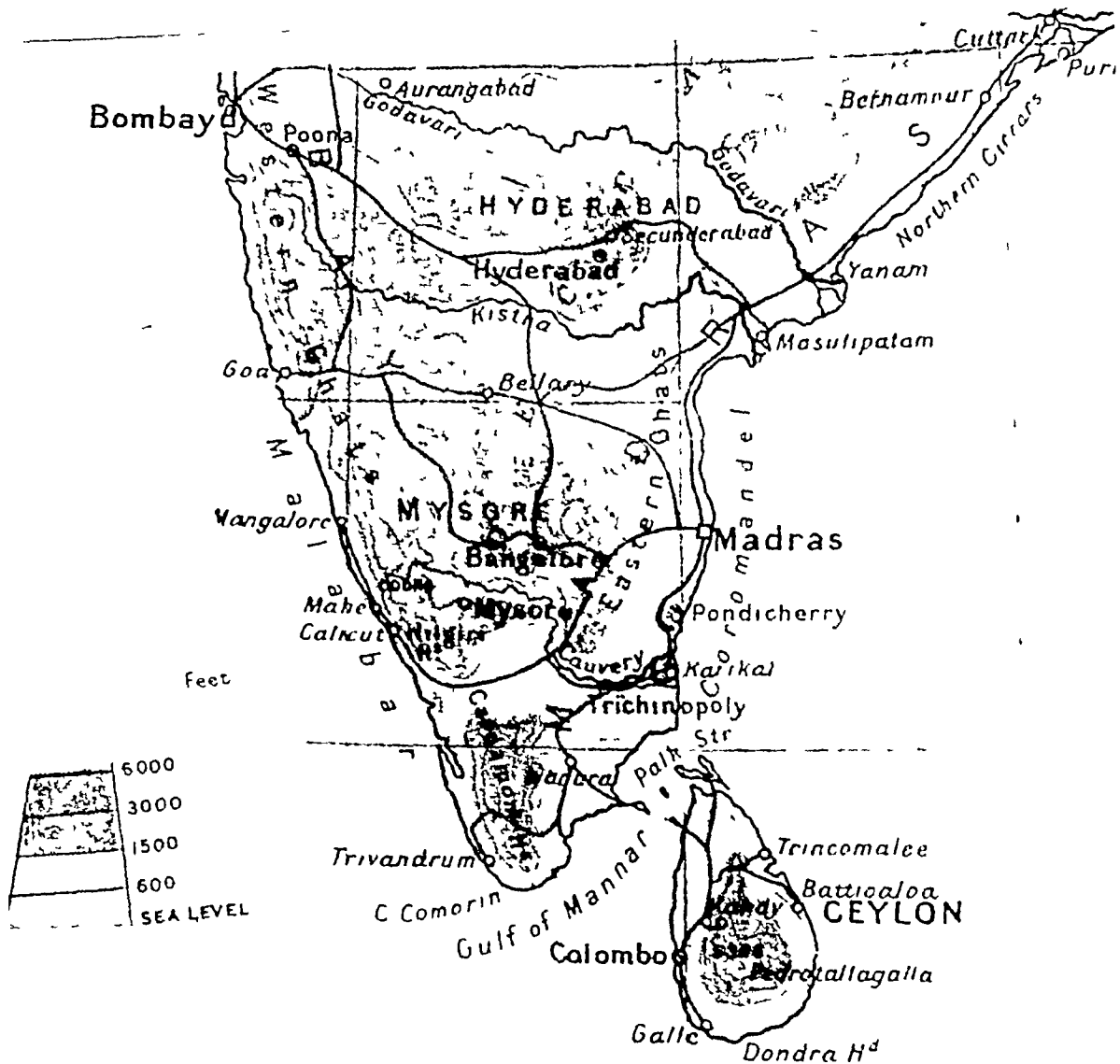
Map 1 shows the main physical features of Peninsular India. From west to east we have first the narrow lowlying West Coast plain, next a range of hills, the Western Ghats, running almost continuously from north to south parallel to the coast. They reach their greatest height, 8,500 feet, in the Nilgiri Hills in the south. Next comes a wide plateau from 3,000 to 1,500 feet high sloping gently to the east and south-east. In places this plateau ends abruptly as a step or ghaut. These edges with outlying groups of hills form the next feature, the broken chain of the Eastern Ghats which are at their highest, about 5,000 feet, in the Shevaroy Hills in the south and in the Vizagapatam Hill Tract in the north. In several places, as along the great rivers, the continental plateau does not end abruptly, but slopes gently eastwards and merges into the East Coast plain. While this plain runs the whole length of the Presidency it is wide only at the deltas of the great rivers, particularly of the Pennar in the centre and of the Cauvery in the south.

These physical features have an important bearing on climate, for, although it is true that in South India as elsewhere latitude and altitude play their part, particularly in the first half of the year from the cold weather months of January and February to the hot weather months of April and May, yet it is mainly the position of an area with regard to the geographical features mentioned that determines the climate for the second half of the year—June to December. For, it is the geographical position that determines the amount of rain that will be received from one or other or both of the two monsoons that blow during this time, and so determines both the temperature and humidity at these seasons.

The South-West Monsoon blows strongly from June to September and affects the whole of India. But while the West Coast gets enough rain to give it particularly a second cold weather, the East Coast plain and the more eastern parts of the plateau get comparatively little rain and remain suffering from the heat

This is largely because the Western Ghats and the continental plateau rob the South-West Monsoon of most of its rain before it reaches the East Coast

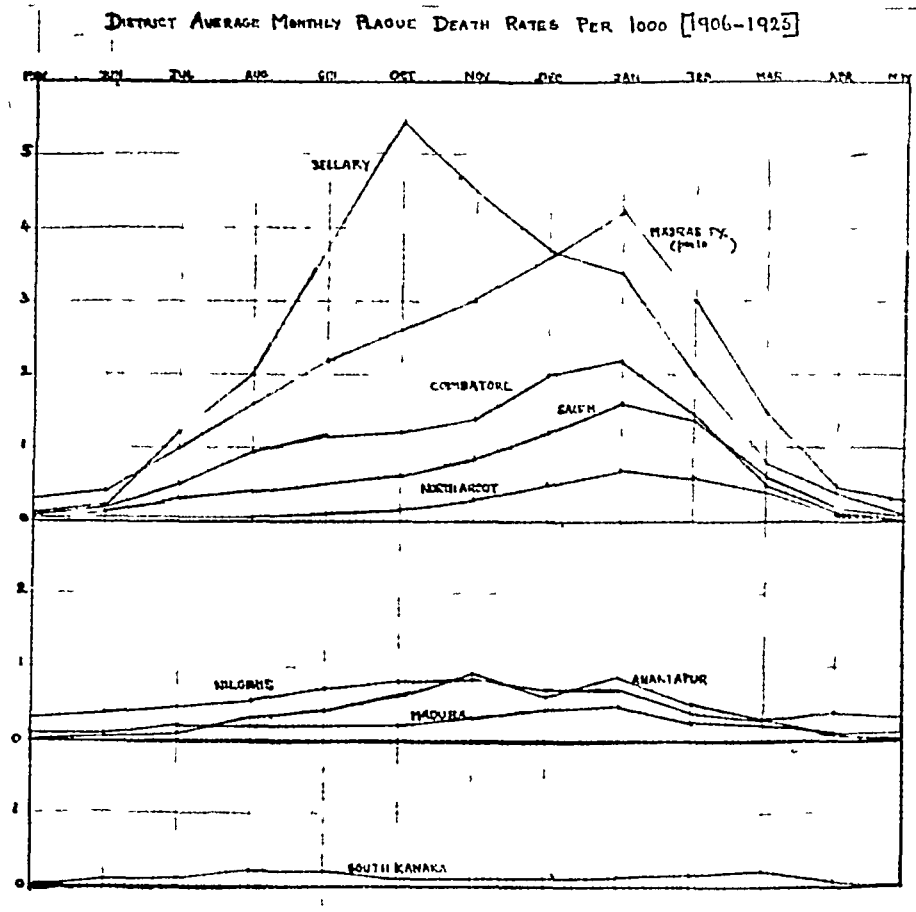
MAP 1



The effect of geographical position in determining local climate and so affecting factors influencing the severity of plague is well seen in the Chart that gives the seasonal curves for plague mortality in the districts most affected by plague(2) Plague increases with the onset of the South-West Monsoon in June and July and the curve then either ascends to an early peak in October—a South-West Monsoon peak, as in Bellary which is the most western of the main plateau districts, or goes

on rising to a later peak in January, a North-East Monsoon peak, in the more southern and eastern districts like Coimbatore, Salem, North Arcot and Madura. Anantapur, a plateau district east of Bellary, shows both peaks, the Nilgiris show the first rise which is maintained as a plateau until January. South Kanara in the West Coast shows a South-West Monsoon rise and then a cold weather rise from January to March—it does not get the North-East Monsoon. The curve for the

CHART



Presidency as a whole, which is labelled 'Madras' and which is on a scale ten times smaller than the others, shows that the rise due to the first monsoon is increased by the second or main monsoon

Rodents.

Of the 17,246 rodents caught as many as 16,607 were *R. rattus* and only 294, or about 1-60th of the whole, were *R. norvegicus*, the rest were a few bandicoots

(*Bandicoota indica* and *malbarica*), musk rats (*Pachyura cerulea*), mice (*Mus dubius*), gerbilles (*gerbille latera curvum*) and the rat *Gunomys loh*

Rattus rattus—Records of the exact variety were unfortunately not kept in every individual survey, but in those that were kept which affect $\frac{1}{2}$ of the total catch, the brown-bellied variety definitely preponderated forming as much as 88 per cent. This preponderance was general and quite irrespective of locality, but it was noticed that when 'field rats,' i.e., rats caught in fields on the outskirts of towns and villages, were *R. rattus*, they were always of the white-bellied variety. So it looks as if the white-bellied were the wild and the brown-bellied the domestic variety. In the Madras Harbour area (and in no other situation) a few rats were obtained whose ventral as well as dorsal fur was jet black, some others here had a rust red dorsal fur with a lighter red ventral fur. The flea indices here and in the reports published all refer only to *R. rattus*, and in this paper, unless otherwise specified, the term 'rat' always refers to *Rattus rattus*.

Rattus norvegicus—These were found mainly at Bezwada a town at the head of the canals of the Kistna delta. A few were also found at Tanjore which is at the head of the Cauvery delta, at Berhampore and at the ports of Calicut and Cochin. So this species is of no importance in South India. The average flea index was 6.8, as compared with 4.9 for *R. rattus*.

Bandicoots—These were common not only in fields but also near houses, only a few were caught as the traps were rather too small for them.

Gunomys loh—This was found only in the Nilgiris where it is the common field rat and where it also freely invades houses. It seldom entered traps. Break-back traps were found more suitable.

Rat density—This varied greatly, from as low as 5 in Ootacamund to 145 in Tirumalai, but as seen from Table I, column 3, it was usually between 20 and 70.

These figures were affected not only by real differences in density, but also by differences in the habits of rats, thus, it was noted that, though plentiful, they were difficult to catch at Yercaud in the Shevaroy Hills and in Ootacamund in the Nilgiri Hills. As usual, the rat density was higher in Bazaar areas than in other parts of a town. The highest figure was 188 in Nellore bazaar, a grain traffic centre. There is no apparent association of density with season but this may be due to small seasonal differences being marked by great local differences.

Rat replenishment rate—This is given in Table I, column 4, for those places in which the requisite data were noted. The replenishment rate(3) is the figure obtained by multiplying the percentage of pregnant females to total rats by the average number of foetuses and dividing by 16 which is the number of days pregnancy in rats is visible to the naked eye.

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Rat movement—Evidence that the movement of rats is usually rather restricted, as has been reported by other observers, was given in the reports for Bellary Cantonment, Harpanahall, Nellore, Tuticorin and other places which showed how

TABLE I

Gross data for rats (Rattus rattus) and fleas in the areas surveyed

Area	Month of survey	Total rats	Rat density	Rat replenishment rate	Total fleas	SPECIFIC FLEA INDICES WHOLE AREA		
						X <i>astia</i>	X <i>braziliensis</i>	X <i>cheopis</i>
Mangalore	March	402	35	4.7	2,051	1.3	1.1	3.3
Calicut *	February	480	24	3.0	2,792	1.5	0.4	3.5
Cochin *	January	169	21	3.0	413	1.7	0.4	
Harpanahall	December	881	77		5,591	0.4	0.2	5.7
Hospet	December	476	43		3,090	1.5		5.0
Bellary	October	869	51		5,287	3.1		2.9
Guntakal	January	330	45		1,365	2.8		1.4
Proddattur	October	129	20	5.4	661	5.1		
Cuddapah	September	481	53	6.1	1,955	4.1		
Nellore	April	1,047	128	5.0	3,398	2.7		0.9
Hosur *	September	375	32	2.4	5,407	1.5	8.7	4.1
Madanapalli	November	138	49	11.3	1,395	3.3		6.7
Trupattur *	April	141	?		298	0.6		1.8
Chittoor	October	330	57	10.7	1,537	3.7		0.9
Vellore	April	116	36	5.2	542	3.1		1.1
Tirupati	May	338	82		1,167	3.5		
Madras I	July	1,025	30	5.3	2,766	2.55		0.15
„ II	December	353	35	4.2	2,448	6.1		0.7

* Rat epizootic at the time of survey

TABLE I—*concl'd*

Area	Month of survey	Total rats	Rat density	Rat replenishment rate	Total fleas	SPECIFIC FLEA INDICES WHOLE AREA		
						<i>X. astia</i>	<i>X. brasiliensis</i>	<i>X. cheopis</i>
Coimbatore	January	331	31	5.6	2,057	0.5	1.6	4.0
Salem	February	437	27	7.0	1,697	1.7		2.8
Trichinopoly	January	454	41	5.8	2,895	6.0		0.1
Tanjore	April	756	65	3.8	2,405	3.3		
Negapatam I	June	466	30	3.2	1,680	3.6		
„ II	January	226	47	6.7	1,316	5.8		
Kumbham Valley*	November	752	13	5.4	4,920	3.6		2.4
Dindigal	January	168	13	3.4	1,221	4.8		2.3
Madura	February	1,809	62	3.8	7,948	3.7		0.6
Tinnevely	September	639	67	4.9	2,168	2.8		0.5
Tuticorin	October	526	34	5.3	2,752	3.3		2.0
Nilgiris	May	88	5	4.4	379	0.4	0.6	2.1
Yercaud	March	37	13	3.7	256	1.4	5.5	
Tirumalai	May	200	145		793	4.0		
Berhampore	September	397	60	0.3	1,833	0.9		3.6
Vizagapatam	October	443	53	3.5	2,408	1.5		3.4
Bezawada	November	715	89	3.3	2,638	2.4		0.04

* Rat epizootic at the time of survey

the prevalence of some particular flea species was sharply restricted to certain areas or even streets

Fleas.

82,708 fleas were examined 59.9 per cent were *X. astia*, 33.5 per cent were *X. cheopis*, and 5.9 per cent were *X. braziliensis*. These are the three main species, and, as is evident, *astia* is by far the commonest. At Ootacamund in the Nilgiri Hills, two other rat-fleas were also found, a few *Leptospylla musculi* and many *Ceratophylus ulgeriensis* which were common there and which formed the chief flea found on the local field rat—*Gunomys lok*. This last species of flea was kindly identified for us by Mr. Mohamed Sherif of the Aligarh University. As usual, other fleas such as *Pulex irritans*, *Ctenocephalis canis* and *Echidnophaga gallinacea* were also found.

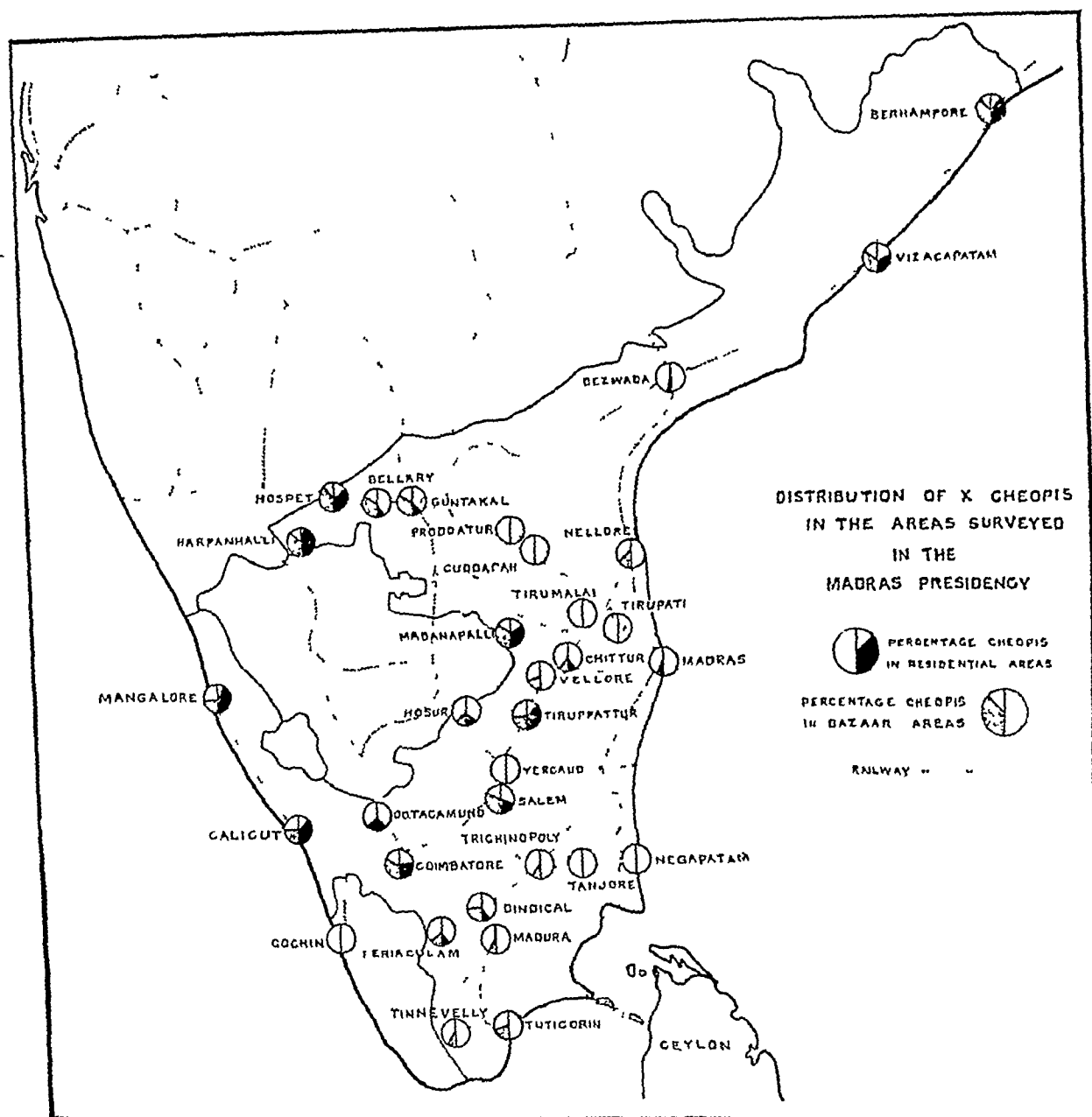
For all representative areas apart from individual villages, etc., the general flea index varied from 2.1 to 14.2, but as seen from Table I, in all but two places it varied from 2.1 to 7.2. The average was 4.9. The variations in this index from one place to another were partly due to seasonal changes. This is readily seen if the indices be arranged in order when it will be found that nearly all the larger indices refer to places surveyed between September and January, i.e., in the cooler and more humid months that correspond with the months where the seasonal plague curves of the Chart are high. For general areas the index for *astia* varied from 0 to 11, for *cheopis* from 0 to 6.7, and for *braziliensis* from 0 to 8.7. In particular localities these were exceeded. The highest indices found were 11.3 for *astia*, 17.6 for *cheopis* and 14.2 for *braziliensis*. In order to simplify the problem of the relative importance of the two main species we will first get rid of the complication introduced by the presence of *braziliensis* in a few places.

The distribution of *braziliensis*.

This was the chief flea found in Hosur and Yercaud and was moderately abundant in Coimbatore and Ootacamund. The situation of the places named can be seen from Map 2. Hosur is on the Mysore plateau, Yercaud is on the Shevaroy Hills not far from this plateau on the south-east, Coimbatore is on the lower plateau south of the Mysore plateau, while the Nilgiris are close to the Mysore plateau on the south-west.

In the other places where it was found, it appears to have been imported with grains into commercial areas, but to have apparently permanently settled only at Harpanahalli on the north of the Mysore plateau, and in the West Coast towns of Mangalore, Calicut and Cochin with their humid climate. Two species were found in Madras in a ground-nut godown and one at the Trichinopoly railway station which suggests the method of importation and reminds us that the process is continuing. To conclude, the chief habitat of *braziliensis* appears to be the Mysore plateau and adjacent similar areas. In the absence of an extended survey of the Mysore plateau we have not enough evidence as to whether it is indigenous.

MAP 2.



The distribution of *astia* and *cheopis* as affected by importation and differences in the local environment.

We prefer to discuss the action of these two factors before considering the association of species distribution with climate and with plague, because, as we shall see, by doing so we shall be in a better position to deal with these other problems later. In Map 2 the shaded portion of each left semi-circle represents the proportion

of *cheopis* found in bazaar areas, by which we mean all mainly commercial areas, shops, godowns, mills, etc., with residences in the immediate vicinity. Similarly, each right semi-circle refers to what are mainly purely residential areas. The exact figures which these diagrams represent can be found in Table VI. The first point to note is that there are very many places which show great differences between the proportions of *cheopis* found in the two contrasted areas. Full details of these differences have been given in the individual reports. They are due to the action of the two factors mentioned. As regards *importation* we have ample evidence to show that whereas *astria* is an indigenous species (and perhaps *braziliensis* too) *cheopis* has been and is being imported into new territories hitherto occupied only by *astria* or by *astria* and *braziliensis*. A summary of the more important evidence for this is as follows —

(a) Whereas *astria* was found everywhere in widely different localities, the distribution of *cheopis* was more restricted. As we shall see, *cheopis* favoured moist and cool climates, but even so it was absent in some areas which seem suitable climatically. This not only suggests that it was absent because it has yet failed to be introduced, but also suggests that, in the places where it is fairly common, it has been introduced in comparatively recent times. Climatically suitable areas into which *cheopis* has not yet penetrated are as follows —

Yercaud 4,500 feet, a hill station had only *astria* and *braziliensis*

Tirumalai 2,800 feet, a place of pilgrimage on an isolated hill in the east, had only *astria*

Bellary Cantonment 1,500 feet. Except in one grain store only *astria* were found

Cochin On the West Coast had only *astria* and a few imported *braziliensis*

(b) There were many towns where from climatic or other reasons *astria* was practically the only flea, and *cheopis* practically non-existent or very scanty, yet in these towns *cheopis* was found in places like import godowns, railway stations, etc. Instances are as follows —

Cuddapah 100 per cent *astria* but one 'loose' *cheopis* was found at the railway station

Nellore Residential areas 99·7 per cent *astria*. Bazaars importing grain from Nizam's dominions had 44 per cent *cheopis*

Vellore Residential areas 100 per cent *astria*. Bazaar 22 per cent *cheopis*

Trichinopoly 100 per cent *astria* in the main area. But some *cheopis* were found in the central grain godown

Madras 100 per cent *astria* in the main area. Perambur cotton mills 90 per cent *cheopis*. Harbour import rice godowns 33 per cent *cheopis*

Town import godowns near harbour 1 per cent *cheopis*

Tuticorin Residential areas 99·7 per cent *astria*. Cotton godowns 74 per cent *cheopis*

Grain godowns 42 per cent *cheopis*

General bazaar 3 per cent *cheopis*

Madura See Table II where the specific indices for *cheopis* and the relative proportion of *cheopis* show the same definite descending order as we get away from areas importing cotton and grains

TABLE II

Flea distribution in Madura Town (Jan -Feb)

Locality	Total fleas	General flea index	<i>cheopis</i> index	<i>astia</i> index	Per cent <i>cheopis</i>	Per cent <i>astia</i>
Madura Mills	532	10.9	9.8	1.1	90.2	9.8
Grain godowns	713	6.2	3.3	2.9	53.9	46.1
Houses near grain godowns	304	5.1	1.1	4.7	22.0	78.0
Grain bazaar	984	3.2	0.4	2.8	12.9	87.1
Houses near grain bazaars	2,744	4.1	0.1	4.0	2.3	97.2
Houses away from bazaars	535	5.5	0	5.5	0	100

TABLE III

Towns showing great local differences in the distribution of X astia and X cheopis

Town	SPECIFIC INDICES <i>X astia</i>		SPECIFIC INDICES <i>X cheopis</i>		PER CENT <i>X cheopis</i>	
	Bazaar	Residences	Bazaar	Residences	Bazaar	Residences
Bellary	2.1	4.3	4.2	1.5	67	25
Guntakal	2.3	2.8	4.4	1.0	66	26
Salem	1.2	2.3	2.4	1.8	66	44
Dindigal	3.8	6.1	3.57	1.3	50	20
Tinnevely	3.0	2.3	0.7	0.04	18	2
Tirupattur	0.5	0.8	0.6	2.5	50	77
Calicut	2.9	0.9	3.4	3.5	47	80
Mangalore	1.5	0.56	2.1	4.3	42	88

(c) In addition to the places just mentioned there were several other towns (see Table III) in which the relative proportion of *cheopis* was much less in residential than in bazaar areas. That this was the rule is probably due not only to the invasion of commercial areas by *cheopis* and its slow spread therefrom into domestic areas, but also to the other factor of differences in environment. We refer to differences in the construction of houses, their cleanliness and degree of crowding together and differences in rat-nesting conditions, etc., for insanitary surroundings seem to favour *cheopis* to a greater extent than they favour *astia*. This factor will work in the same direction as that of importation, when the conditions for *cheopis* are less favourable in residences than in bazaars, by hindering their spread to residences, but will work

in the opposite way when the reverse is the case. This is probably one reason why the last three towns show a reversal of the locality *cheopis* proportions. In Calicut it was noted that the housing conditions of many domestic quarters were decidedly worse than those of the houses in the bazaar. The same conditions held good for fishermen's huts in Mangalore. Poor general housing conditions were also observed in Tirupattur. Another reason for the high proportion for *cheopis* in residences in Calicut and Tirupattur was the existence of plague in them. Further it should be noted that the bazaar areas of these towns already show *cheopis* proportions near 50 per cent, so if the figures represent the usual conditions it is possible that in these towns there has been no reversal of the usual process—the spread of *cheopis* from bazaars to residential areas, but simply a quickening favoured by favourable conditions in the residences.

The specific indices for these towns and for Madura show, that in places where the *cheopis* index is higher in one part of the town than in the other, the opposite tends to hold good for *astria*, so though there are differences in the general flea indices for the two sorts of areas, there is also a change in the relative proportion of the two species. This points to a definite struggle for existence between these two species.

(d) As a continuation of the general rule of diminishing *cheopis* infestation as we proceed from the centre of a town outwards, we may adduce the evidence of the few rats that were caught in traps set in the fields. These rats were *Rattus rattus* mainly of the white-bellied variety. On these no *cheopis* at all were found. Thus outside Bellary and Hospet which have *cheopis* rates of 57 per cent and 78 per cent, 7 rats were caught in fields from which 10 *astria* and no *cheopis* were obtained. In the Kumbum Valley with 37 per cent *cheopis*, 13 rats caught in fields yielded 3 *astria* and no *cheopis*. Outside Hosur where the *cheopis* rate is 28 per cent, 3 rats yielded 10 *braziliensis* and no *cheopis*. Outside Tuticorin which contains *cheopis* in its godown areas, 9 gerbilles were caught which yielded 13 *astria*. Some bandicoots at Bellary, Guntakal and Ootacamund showed the same phenomenon of *astria* only, though usually these rodents (which freely enter towns) showed *cheopis* as well.

As regards *astria*, because it is already so widespread, indirect evidence, as in the case of *cheopis* does not suffice to show importation, but of course it is also being freely exported from and imported into different places as much, if not, more than *cheopis*. Thus at Tanjore the examination of imported grains before entering a godown yielded a dust from which *astria* fleas were later hatched out.

Conclusions.

(1) While *astria* appears to be so widespread as to allow us to call it indigenous, *cheopis* appears to be a flea that is still invading new territory.

(2) It is inadvisable to set much value on small differences between towns, for it is very evident that the flea indices for any one town will usually vary with the proportion of the rats caught in the bazaar areas.

(3) Differences between towns in the degree of infestation of rats with fleas and in the relative proportion of species present, are probably partly due to variations in the two factors of importation and general environment that cause differences between parts of the same town

(4) What is already well known and what has been brought out in the individual reports, is the importance of grain trade in the transport of fleas

(5) A conclusion not so well known and that has never been previously stressed so far as we know, is the very great importance of the *cotton* trade in the transport of fleas, particularly of *cheopis*. This has already been noted in the Reports but the evidence may be conveniently summarized here, for we think that in these parts of India this trade is as important if not more important than the grain trade in the dissemination of *cheopis*

Influence of the cotton trade.

The importance of the cotton trade in the dispersal of *X cheopis* was made apparent by the Madras survey. Here *X cheopis* were found only in two localities, in the harbour godowns stocking Rangoon rice, and in the cotton mills at Perambur where they formed over 90 per cent of the total fleas. Evidently they were imported from the western hinterland from where the supply of cotton was obtained and which were known to be infected with *X cheopis*.

As shown by Table IV, similar observations have also been reported in several towns where premises connected with the cotton trade, such as godowns, pressing and ginning factories and spinning and weaving mills, showed not only practically invariably a high general flea index but also usually showed a much higher figure for the proportion of *cheopis* than for the rest of the towns. That importation takes place through cotton is clearly shown by the findings in Tinnevely and Tuticorin. In Tinnevely the grain trade is small, and Sahyar street, which is the centre of the grain trade, did not show any *X cheopis*. On the other hand, the centre of the cotton trade—the town bazaar—showed the presence of *X cheopis* in considerable numbers, 296 out of 1,252 total fleas thus collected. Again, out of 7 *cheopis* obtained in residential areas, 6 were collected from the houses of weavers who obtain their yarn from such *cheopis*-infected places as Hubli, Dharwar, etc., in the Bombay Presidency.

It can be observed from Table IV, that the cotton mills on the whole show both a high *cheopis* percentage and a high *cheopis* index, 3 to 4 times the average. It is probable that this high index is maintained by frequent importations, for, as will be seen from the discussions on climate in some of these places such as Madras, Madura, Tuticorin, *cheopis* is absent or scarce in the ordinary parts of the same town. That such importation is irregular is brought out by the fact that in some mills in Madras and Madura *cheopis* are not found at all, or only exist in very small numbers. An explanation for this anomaly is not

TABLE IV

Fleas in cotton mills and godowns

Town	Month of survey	FLEA INDICES IN WHOLE TOWN			FLEA INDICES IN COTTON PREMISES			CHITOPIS PERCENTAGE	
		General	asha	cheopis	General	asha	cheopis	Whole town	Cotton premises
<i>Tinnevely</i> —									
Town bazaar	September	34	29	05	46	35	11	15.5	24.4
Tuticorin	October	52	33	19	84	21	62	36.4	74.4
Dindigul	"	72	48	24				33.0	55.2
Coimbatore	January	61	05	40	135	02	128	64.0	94.0
<i>Theni</i> (in Kumbum Valley)	"	69	48	20				28.8	93.2
<i>Madura</i> —									
Madura Mills	"	43	37	06	109	11	98	15.0	90.3
Other Mills	"				53	52	01		15
<i>Madras</i> —									
Perambur Mills	December	68	61	07	65	03	62	10.7	95.6
Choolai Mills	"				113	113			
<i>Gaihaut</i> —									
Kallai Mills	January	54	15	35	176	0	17.6	59.5	100.0
Wist Mills	"				54	44	10		18.5

TABLE V
Loose flea experiments—Tulacorn

Places	Number of traps with defleaed rats	Number of loose fleas caught	General loose flea index per trap	General attached flea index	X <i>astia</i>				X <i>cheopis</i>			
					Number of loose <i>astia</i>	Loose <i>astia</i> index per trap	Attached <i>astia</i> index per rat	Ratio loose index to attached index	Number of loose <i>cheopis</i>	Loose <i>cheopis</i> index per trap	Attached <i>cheopis</i> index per rat	Ratio loose index to attached index
Loose cotton godowns	12	160	13.33	12.42	32	2.66	2.05	1 1 3	128	10.66	10.37	1 1 0
Pressed cotton godowns	20	24	1.2	5.6	13	0.65	3.33	1 0 2	11	0.55	2.22	1 0 2
Gram godowns	29	78	2.7	6.3	43	1.48	3.75	1 0 4	35	1.21	2.5	1 0 6
Dhal godowns	11	2	0.18	1.8	1		1.77		1		.	
Houses	6	0		7.9	0				0			

apparent, but there is some reason to believe that importation depends on seasonal factors, as to whether the supply of cotton to a mill is from the summer or winter cotton crop. In the case of the latter, the infestation with *cheopis* is likely to be considerable.

Cotton seems to be a particularly suitable material for the dispersal of fleas because of the shelter from mechanical damage and from drying that it affords, and further the highly humid atmosphere of cotton mills neutralizes the disadvantages of an otherwise unfavourable climate. Cotton dust sweepings seem to afford excellent shelter for fleas when they are away from their rat-hosts and so help flea breeding. This was demonstrated by the 'Loose flea experiments' which were conducted during the survey in several places. These experiments were designed firstly to find out if there was any gross infestation of grain, etc., with fleas, and secondly to investigate the comparative rôles of cotton and varieties of grain in their dispersal. The technique adopted was discussed in detail in Report No. XI for Tinnevely. It was observed that loose flea densities for both *astia* and *cheopis* varied greatly in godowns stocking different materials. The highest densities in all places were in loose cotton godowns. This is well exemplified by the Tuticoin experiments given in Table V, in which, as can be seen, not only were the loose flea indices per trap highest in the loose cotton godowns, but their ratio to the specific indices for the same places were much higher than elsewhere. The influence of cotton mills on the general rat-flea population of a town is shown by Table III where all the first five towns, giving much higher *cheopis* percentage in commercial areas than in residences, possess local ginning and pressing cotton mills.

It is thus evident that the cotton trade is a potential source for the introduction of infection into a locality. Direct evidence for this is an outbreak of plague in 1929 in Kotturu in Bellary District. An extract from the report of Dr. Sri Ramulu, the Chief Plague Officer of the District at the time, reads as follows—

'The infection was brought into the town through raw cotton which probably contained infected rat-fleas.

'The first rat-fall occurred in the cotton mill—cotton having been brought to the mill a month previously from two infected places some 6 miles away. The first case occurred among the workmen residing in the compound of this mill. The town which is situated some 3 miles away from the mill was then infected. The first rat-fall occurred in a shop belonging to the owner of the above mill who had probably removed some material there from the mill.'

Seasonal changes.

The ideal data would be those obtained from places representative of different climates which had been surveyed in the same localities all the year round, or at least in the four chief seasons of the year. Such ideal data were not obtained in this rapid general survey. The King Institute is now doing surveys throughout

the year in three representative areas, so we hope to be in a position a year hence to describe seasonal changes more accurately than we can at present. Even so the few data already obtained will be of interest.

There is some direct evidence of the effect of seasons in the repeated surveys of Madras and Negapatam. The first surveys were in June, July and August and the second surveys in January and December. On the whole the *astia* index for residential areas was higher in the cold weather than in the hot, e.g., in Madras 5.8 as against 4.3. There was still a greater increase in the *astia* indices in the chief godowns, e.g., in Negapatam 7.24 as compared with 4.4. *Cheopsis* which had been found in these places only in the Madras harbour godowns and in a cotton mill also showed a rise (a greater rise than *astia*) in the cold weather, e.g., for the cotton mill 6.2 as against 1.9 in the hot weather, and for the harbour 3.9 as against 0.3. These *cheopsis* areas are very subject to importation which might account for some of the cold weather rise, but it is doubtful whether it would account for the whole rise. So we may conclude that on the East Coast plain both *cheopsis* and *astia* tend to be more plentiful in the cold weather at the end of the North-East Monsoon than in the hot weather and early South-West Monsoon.

Next we have the evidence given by two surveys of the small town of Uthamapalayam in the Kumbum Valley about 1,000 feet high on the west of the southern part of the main plateau which was surveyed in December 1928 and January 1929. It has recently been again surveyed in June 1930 by Dr. George in another Inquiry under Major Hesterlow, I.M.S., the Director of Public Health, Madras, to whom we are indebted for the results of the second survey. The two surveys show a reversal of the relative proportions of *astia* and *cheopsis*. Whereas in the first cold weather survey these formed 28 and 57 per cent respectively of all fleas, or disregarding *Echidnophaga gallinacea*, formed 33 and 67 per cent respectively, in the hot weather the proportions were 64 and 36 per cent.

Unfortunately these results were complicated by a severe epidemic of plague in the first survey when the rat density was only 1.3 as compared with 9.3 in the second. This affected the general flea index which was 8.18 in the first survey and only 4.44 in the second. The specific indices in the first survey were 2.3 for *astia* and 4.69 for *cheopsis*. In the second survey the indices were 2.83 for *astia* and only 1.61 for *cheopsis*. Thus there was a diminution of the *cheopsis* index in the second survey to one-third of its former value. We doubt whether this change in the percentage rates is quite typical of the South Indian plateau. Plague probably disturbed the species ratio in the first survey, to the advantage of *cheopsis* unduly exalting its index at the time, which is a phenomenon that was noted elsewhere as in the union of Narayanadevagi, where the *cheopsis* index alone rose—see Table IV in the report for the Bellary area. This increase of *cheopsis* in the presence of plague may be only an apparent increase due to greater activity or may be a real increase. A lethal action of plague on *astia* has been suggested. This may be the

reason for the low *astria* indices found at Tirupattur (0.6) just after a plague epidemic and at Cochin (2.15) on the West Coast during an epizootic of plague. Whatever the causes of such differences it reminds us that caution must be used in comparing one place with another in the absence of data for both places for all seasons and in the presence of plague in either.

Finally, we have indirect evidence suggestive of the general similarity of seasonal changes in *astria* and *cheopsis*, in the fact, that in places like Madras, Negapatam, Cuddapah or Cochin, where, as we shall show, plague must have been carried almost entirely by *astria*, the seasonal prevalence of plague on the few occasions it has occurred is the same as in towns where plague is carried mainly by *cheopsis*. This of course may be due entirely to the effect of season on only the other factors in *plague transmission* apart from any action on flea prevalence as well, but it is not very likely.

Some general considerations in determining the association of species prevalence with climate.

We will first briefly consider each factor that affects flea prevalence and how its action can be diminished in order to better estimate the effect of climate.

Repeated importation may succeed in establishing a colony of a species in only moderately favourable surroundings. This is evident from the facts already discussed. It can be partially discounted by restricting ourselves to figures for *residences*, for of course it is the commercial areas that are most subject to importation.

What we may call the 'general factor,' or environment, apart from temperature and humidity, as it affects flea breeding conditions and facilitates the contact of fleas with rats, largely influences the general flea index and not only that, but as we have seen, also influences the relative prevalence of *astria* and *cheopsis*. This factor also may partially be discounted by restricting ourselves to figures for residences, which, on the whole vary somewhat less among themselves in this respect than do commercial areas. Further, to the extent that the general environment affects the absolute size of specific indices, it can be discounted by considering the *ratios* of the specific indices or more conveniently the species percentages.

The *season* at which a survey is done influences the figures for both the absolute or the relative prevalence of a species. Once its effect is fully known, then of course it can easily be discounted, but in the absence of this knowledge, as here, it can still be partially allowed for. To the extent that it influences the absolute prevalence of species, it can be eliminated, as in the case of the general factor, by considering only the relative percentages for species. Further, its effect in a survey such as this can be diminished if a sufficient number of places are surveyed, for, unless the peaks of the seasonal prevalence curves of *astria* and *cheopsis* are widely separated—for which there is no evidence—a large number of different surveys, as here, would

ensure that many of them are done at a season when the several curves for the two species are approximately parallel and then ratios are near the general average for the ratio of the two species

The presence, or recent existence, of *epizootic plague* not only tends to increase the general flea index by diminishing the number of rats, but also, as stated, tends to markedly increase the relative preponderance of *cheopis* over *astria*. This latter action may be entirely due to the lethal action of plague on *astria* as has been suggested, but it may also be due to a greater activity on the part of *cheopis*. The only way to discount this factor is to note the presence of recent epizootic plague.

From these considerations it will be apparent that in order to find the effect of different climates over the whole year in determining the relative prevalence of flea species, we shall do best if we consider the *species percentages* for *residences*, for then we shall diminish as far as possible the disturbing effect of the first three factors mentioned. The *specific indices* at the same time will still have to be considered in order to make sure that we are not dealing with exceptional cases in which the specific indices are abnormally high or low. There has been some controversy in the past over the use of species percentages, but we think it is because the individual action of the seasonal factors at work has not been sufficiently discussed, and also because these relative percentages have *alone* been used for discussing the association of species with *plague*. Since when we deal with plague what we would like to have is the exact specific indices at the plague season, figures for the relative species prevalence will not serve unless an indication of the level of absolute prevalence be also given.

The association of the distribution of *astria* and *cheopis* with climate.

Let us examine Map 2 and consider what relation the residential *cheopis* percentages bear to geographical position and climate. The exact data on which this map is based are given in Table VI where the areas surveyed are arranged in order of diminishing *cheopis* percentages. From the map we see that in the humid climate of the West Coast ports, Mangalore and Calcut, *cheopis* was abundant when it existed at all, but, that as an exception, it was absent in Cochin.

Next we see that on the main plateau and plains of the Peninsula east of the Western Ghats, *cheopis* infestation diminishes as we go from west to east or from north-west to south-east and as we go from higher to lower lying land. Thus compare the following general west to east or north-west to south-east 'lines' which are also, on the whole, the lines of diminishing altitude.

(1) The North Mysore—Pennaru River Line—Harpanahalli, Hospet, Bellary, Guntakal, Proddatur, Cuddapah and Nellore.

(2) A central rather irregular line—Hosur, Madanapalle, Tirupattur, Chittoor, Vellore, Tirupati and Madras. It is to be noted that the specific indices for the first two places are much higher than for the others (*see* Table VI).

TABLE VI

Flea species local differences and by climate Areas in order of diminishing cheopis percentage in residences

Area	Month of survey	BAZAAR AREAS				RESIDENTIAL AREAS				Number of months off season	Actual months off season	Altitude		
		General flea index	SPECIES PERCENT AGE			General flea index	Specific <i>astia</i> index	Specific <i>cheopis</i> index	SPECIES PERCENT AGE					
			<i>astia</i>	<i>brazilhensis</i>	<i>cheopis</i>				<i>astia</i>				<i>brazilhensis</i>	<i>cheopis</i>
Harpanahalli	Dec	6.4	0	24.4	75.6	6.3	0.4	5.8	7.1	0.7	92.2	1,572		
Mangalore	Mar	5.1	29.0	29.0	42.0	4.9	0.6	4.3	12.0	nil	88.0	Sea level		
Berhampore	Sept	4.0	23.0	nil	77.0	5.1	1.0	4.1	20.0	"	80.0	79		
Calcutt *	Feb	7.1	41.0	12.0	47.0	4.4	0.9	3.5	20.0	"	80.0	Sea level		
Trupattur *	April		50.0	nil	50.0	3.4	0.8	2.6	23.0	"	77.0	1,233		
Hospet	Dec	6.5	22.2	"	77.8	6.4	1.5	4.9	24.0	"	76.0	1,572		
Madanapalli	Nov	6.6	31.0	"	69.0	10.7	3.5	7.2	33.0	"	67.0	2,250		
Viragapatam	Oct	3.8	31.8	"	68.2	5.2	2.1	3.1	41.8	"	58.0	Sea level		
Combatore	Jan	8.0	10.0	21.0	69.0	4.6	0.4	2.6	10.0	34.0	56.0	1,400		
Ootacamund	May					4.9	0.4	2.4	9.7	14.3	49.0	7,500		
Salem	Feb	3.6	34.0	nil	66.0	4.2	2.3	1.9	55.5	nil	44.5	946		
Kumbum Valley*	Nov	6.8	66.0	"	34.0	4.0	2.3	1.7	58.0	"	42.0	987		
Hosur *	Sept	11.0	3.4	70.1	24.5	15.3	2.1	4.9	13.0	57.0	30.0	2 900		

Guntakal	..	Jan	69	338	ml	66.2	38	28	10	738	ml	26.2	2 or 3?	..	1,432
Bellary	.	Oct	57	333	"	66.7	71	61	10	750	"	25.0	2	Apr-May	1,521
Chittoor		Oct	55	776	"	22.4	42	33	09	79.4	"	20.6	3 or 4?		950
Dindigal		Jan	70	500	"	50.0	60	45	15	79.5	"	20.5	1		922
Tinnevely		Sept	40	816	"	18.4	23	22.5	005	98.2	"	1.8		.	127
Nellore		April	35	615	"	38.5	32	3.29	001	99.7	"	0.3	6	Apr-Sept	84
Cochin *		Jan	25	756	24.4	0	23	23	0	99.2	0.8	0	3	Mar-May	Sea level
Tuticorin		Oct	53	577	ml	42.3	45	45	0	100.0	ml	ml		..	Sea level
Madura		Feb	43	842	"	15.8	45	45	0	100.0	"	"	6	Apr-Sept	445
Yercaud		Mar					70	15	0	21.0	79.0	"		.	4,500
Negapatam	{	June	37	100.0	ml	ml	3.4	3.4	0	100.0	0	"	7	Mar-Sept	Sea level
	{	Jan	63				36	36	0						
Trichnopoly		Jan	51	981	"	1.9	66	66	0	100.0	ml	"	8	Mar-Oct	256
Tanjore		Apr	31	100.0		ml	29	29	0	100.0			6 or 7?		193
Trumalai		May		100.0			39	0.1	0	100.0	ml	ml			2,782
Trupati		May	30	100.0	ml	ml	38	38	0	100.0	"	"	4 or 5?		628
Vellore		April	58	600	"	40.0	48	48	0	100.0	"	"	4	Apr-July	694
Madras	{	July	23	915	"	8.5	41	41	0	100.0	"	"	5 (6)	Apr-Aug	Sea level
	{	Dec	71	877	"	12.3	58	58	0						
Cuddapah		Sept	48	100.0	"		3.4	3.4	0	100.0	"	"	7	Mar-Sept	463
Prodattur		Oct	46	100.0	"		68	68	0	100.0	"	"	6?		509
Bezawda		Nov	39	938	"	1.2	33	33	0	100.0	"	"	3	Apr-June	63

* Rat eps/ootic at the time of survey

(3) The Cauvery River Line—Coimbatore, Salem, Trichinopoly, Tanjore and Negapatam

(4) A Southern Line—the Kumbum Valley, Dindigal, Madura, Tinnevely and Tuticorin

Altitude acts in the same way as westerly position (with which it is generally associated), for, the higher or the more further west a place is, the more rain it gets from the South-West Monsoon

Thus consider in the order of diminishing heights the hill stations of Ootacamund, Yercaud and Tirumalai

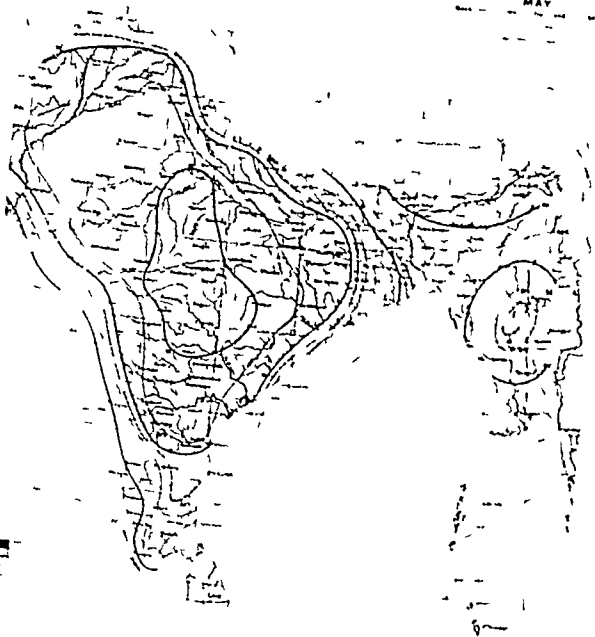
Finally we have the line—Berhampore, Vizagapatam, and Bezwada from north to south in the northern part of the East Coast plain

From Table VI we see that the areas surveyed fall into two sharply distinct groups, the first seventeen with *cheopis* percentages above 20, and the last 16 with *cheopis* percentages below 2 practically all of which are in the south and east. The specific indices follow suit, while they vary from 0.9 upwards in the first group they are all practically zero in the second group. It is these sharp differences which enable us to draw conclusions from these results without a full knowledge of the seasonal changes. The absence of intermediate groups is striking and points to the existence of some critical conditions for climate beyond which *cheopis* does not readily survive. Such must have operated to prevent the colonization of the residential areas of towns in the South-East group. Once these critical conditions are passed we can get differences due to the different degree in which climate favours one species, so this probably partly accounts for the gradation in the first group.

We think that all will agree that the table and the map clearly show a striking association between *cheopis* prevalence and geographical position—in other words, with climate. In trying to see how these marked geographical differences were dependent on climate, we obtained figures for temperature and humidity in the favourable flea season which we took as the four months preceding the peak of the district plague mortality curve. We could find no definite association between these figures and species prevalence. We also examined similar figures for the chief off-season, the hot months of April, May and June, but still could get no definite association. Finally, we considered the duration of the off-season. The map shows that the area of lowest *cheopis* infestation is the central and southern portion of the great East Coast plain between Bezwada and Tuticorin, and that *cheopis* infestation increases in proportion as we go to the west and north of it. This eastern area is the region least affected by the South-West Monsoon, and so the region where the hot weather, instead of ending in June with the burst of this monsoon, is prolonged into September, thus greatly prolonging the off-season for plague and for fleas. This prolongation of the hot off-season in the south-east is clearly shown by Maps 3 to 12 (copied from the Climatological Atlas of India) which give the contours for temperature and humidity from May to September. We hesitate to label any

MAPS 3-6

MEAN TEMPER. TIME OF DAY
MAY

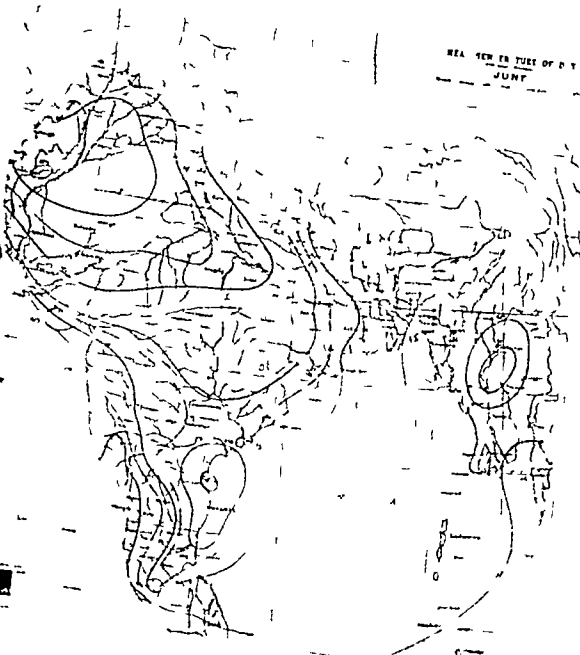


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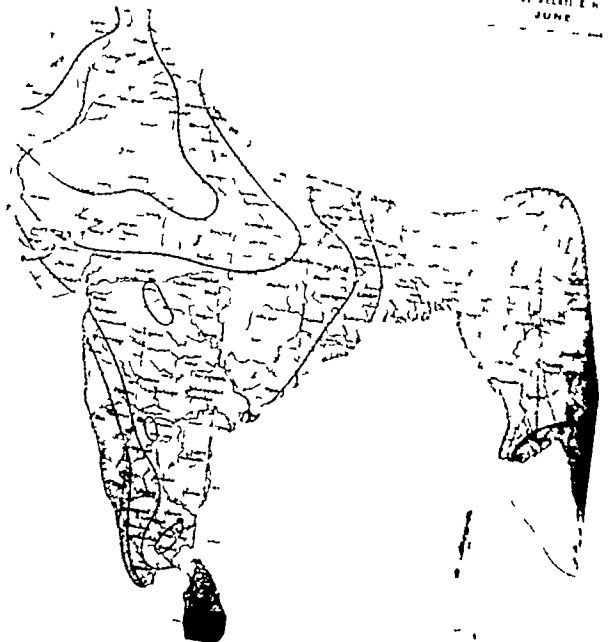
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MEAN TEMPER. TIME OF DAY
JUNE



5

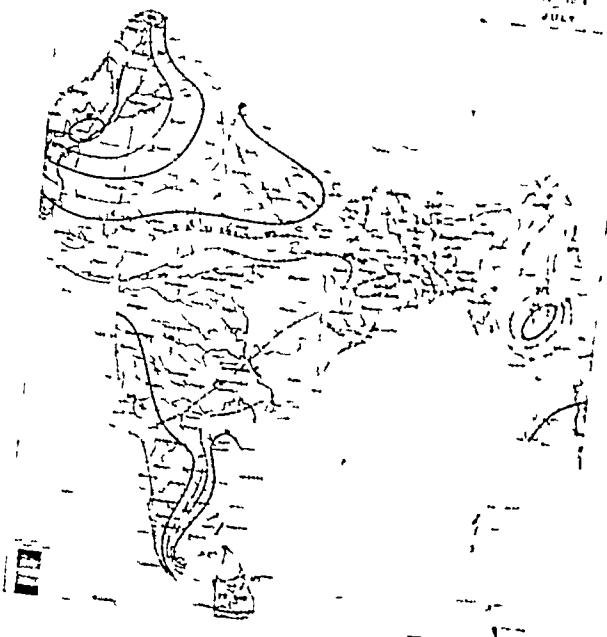
MEAN TEMPER. TIME OF DAY
JUNE



6

MAPS 7-10

HEATH 10, 10, 10, 10
JULY

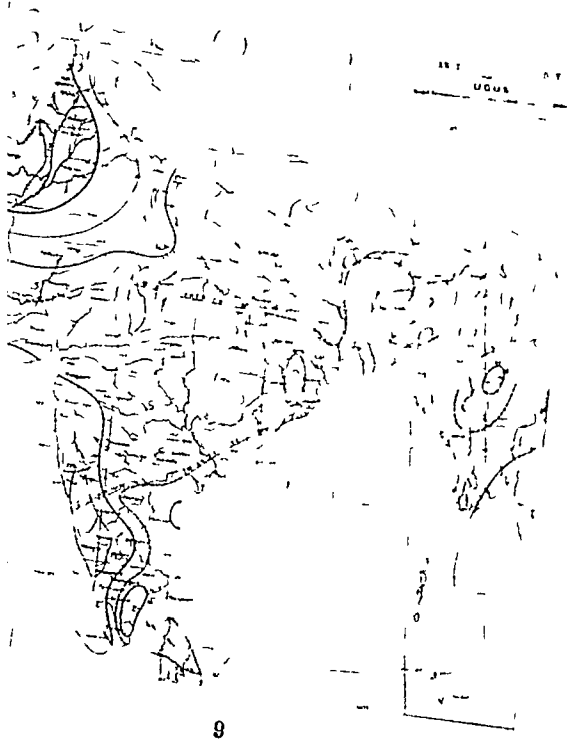


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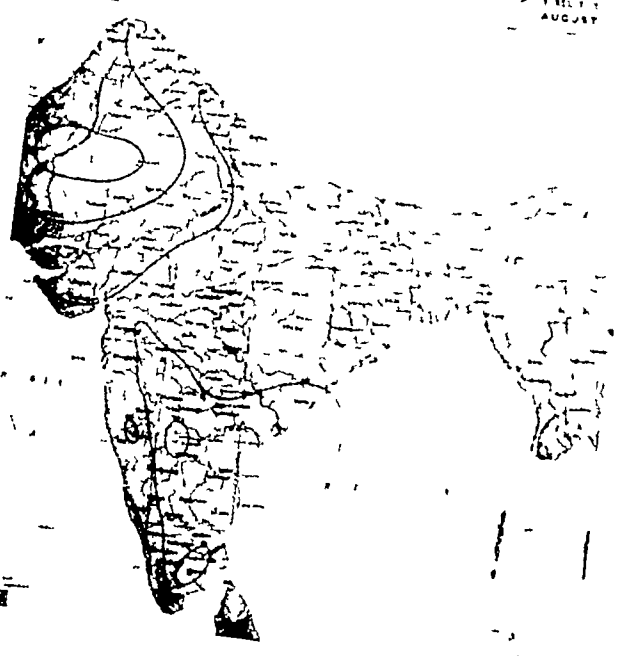
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HEATH 10, 10, 10, 10
AUGUST



9

HEATH 10, 10, 10, 10
AUGUST



10

MAPS 11—12



11.

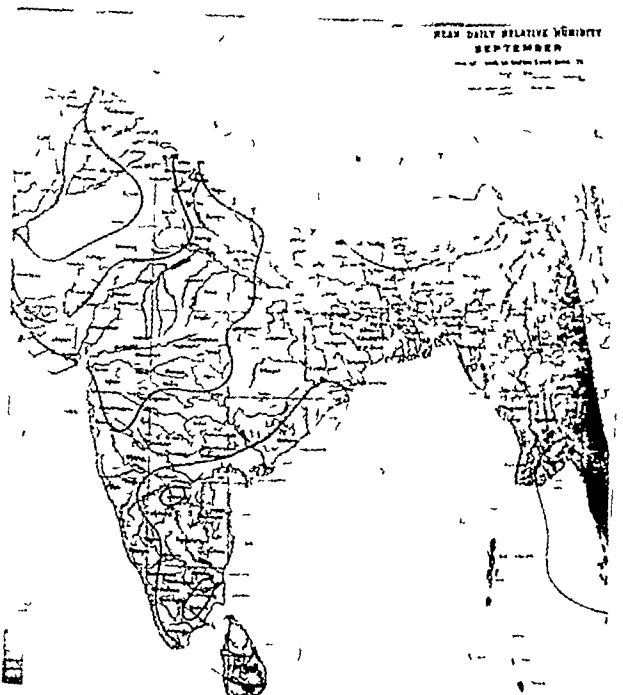


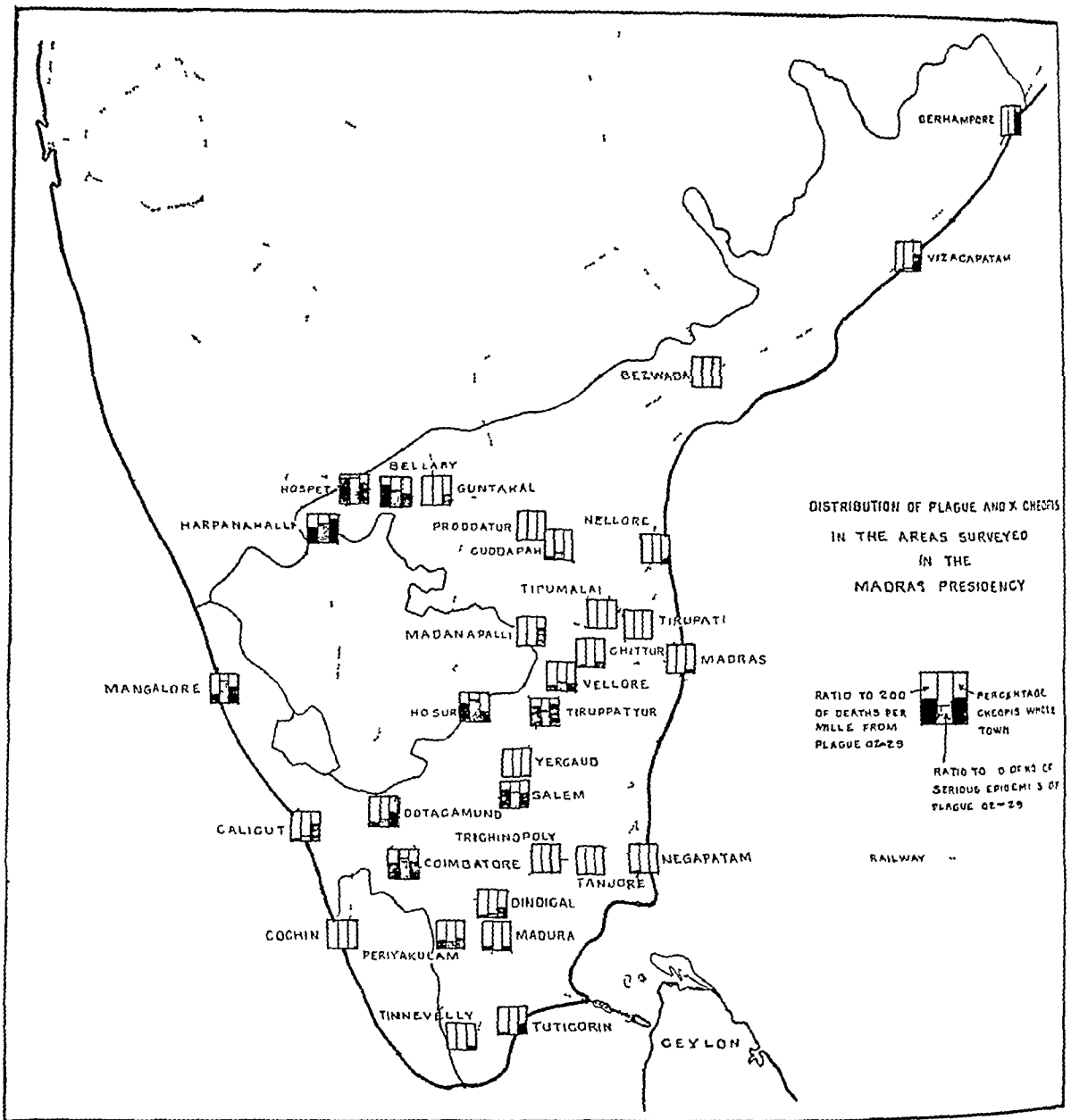
figure as critical, but on the whole we may take a mean monthly temperature above 81°F and a mean monthly relative humidity below 76 per cent as those which mark off the long off-season in the south-eastern area. The maps show too the reason for the abundance of *cheops* in the northern parts of the East Coast plains, for these parts, during the hot weather and South-West Monsoon, get moist winds blowing in from the Bay of Bengal, which amounts in this case, as it were, to a local South-East Monsoon. In the table, note how for all places for which meteorological data are recorded, long off-seasons are invariably associated with low *cheops* percentages. Here the off-season is taken as the period during which the monthly mean temperature at 8 A.M. exceeded 81°F and when at the same time the monthly mean relative humidity at 8 A.M. was below 76 per cent. The figures are based on the averages for the last 3 years. For some places for which no meteorological data are available we have estimated the off-season from the figures for places near-by, all such figures are marked with a query.

Thus we conclude that it is not so much the *severity* of the hot weather in temperature or dryness that causes conditions unfavourable to *cheops*, as the much longer *duration* of unfavourable conditions in the south-east side of the peninsula. This is responsible for the failure of *cheops* to spread in this region despite, as we have shown, much importation. While this is so, we must still remember that favourable local conditions can, to some extent, replace a generally unsuitable climate as in parts of Nellore, Madras and Tuticorin, so the possibility of the spread of this flea in the more insanitary parts of towns must be borne in mind.

The association of species distribution with plague.

Map 13 helps us to see this association at a glance. It is based on Table VII where other relevant details, such as the specific indices, are also given for the towns concerned and on Table VIII, which gives the exact figures for plague deaths year by year. We are dealing now with *individual towns*, and not, as before, with areas grouped round a town. This is in order to let us use the exact figures for plague which have been kindly given to us by the Director of Public Health, Madras. In the map for each town the shaded portion of the column on the right represents the percentage of *cheops* to total fleas, the shaded portion of the column on the left represents the number of deaths from plague per 1,000 of population, during the period 1902 to 1929 up to a maximum of 200 deaths per thousand which would be presented by a shading of the complete column. The dotted areas in the central column represent the number of years between 1902 and 1929, in which deaths from plague exceeded 1 per thousand of the population up to a maximum of 20. Thus this column represents the number of serious epidemics.

MAP 13



As for climate and *cheops* distribution, so here, despite the absence of complete data, there appears to be a very definite association between *cheops* prevalence and the occurrence and severity of plague. This is particularly apparent in some of the west to east or south-east lines from the main plateau to the coast that we have already considered under climate and that should be examined again on this map. Note how the places at the head of the lines are either on or close to the main plateau,

the region of *cheopsis* abundance, and how the ends of the lines, where plague is practically absent, are in the *cheopsis*-free region of the Central and South-East Coast plains, similarly the hill station group of Ootacamund, Yercaud and Tirumalai with plague practically absent in the last two. The West Coast line Mangalore and Calicut show *cheopsis* and plague in the first two but not in Cochin which is free from *cheopsis*—the *asria* index here is low but there was a plague epizootic going on. There are two exceptions to the general rule as can be seen both from the map and from the table. The first is the Central Line—Madanapalli—which has had less infection than one would have expected. It is not on a railway and is quite out of the line of ordinary trade traffic. So the comparative absence of importation of infection is the explanation of its relative freedom. This is probably also partly the explanation why, as our second exception, in the northern half of the East Coast plain, Vizagapatam and Berhampore have had little or no plague despite high *cheopsis* infestation. A further possibility is that, whereas the climatic conditions here are suitable for *cheopsis*, they are not suitable for the other factors in plague transmission.

In Table VII the towns have been arranged in descending order of *cheopsis* percentages for the whole town. The general association of high *cheopsis* percentages and indices with the figures for plague is very evident. Practically the same group of towns is in the lower half of the list as were in Table VI. Most of the differences that exist between places in these two tables are due to the fact that *cheopsis* figures relate only to residential areas in Table VI but to the whole town in Table VII, as is only fitting in dealing with plague. So we may say that the typical *asria* zone is practically plague-free.

What we have to remember, before associating, the association of *cheopsis* prevalence with plague, is that possibly the demonstrated association of plague with *cheopsis* prevalence may be entirely spurious. For it may be, that both plague and *cheopsis* prevalence are directly correlated with climate, and so may appear to be correlated with each other despite the possible absence of any connecting causative factors. Against this there stands out the salient fact that the places mentioned previously which are free from *cheopsis* and where the climate is eminently suitable for plague have had either no plague, or when it did appear, suffered very little indeed as can be seen from Table IX.

Cochin, a port on the West Coast which is exposed to infection from other West Coast parts and from the hinterland and which has a climate similar to Mangalore, Calicut and Colombo which all have plague, has had no plague at all in the British area, and only 14 cases in an epidemic in Mattancherry which adjoins it.

Bellary Cantonment on the main plateau with only *asria* has had only 7 cases of plague in the last 16 years. It adjoins the town of Bellary where both *cheopsis* and plague abound. Being a Cantonment its sanitation is of course better than that of the town but this alone will not account for the vast difference.

TABLE VII

Flea species and plague (Towns in order of diminishing cheopis percentage in whole town)

Town	Human population in thousands (1921)	Month of survey	SPECIFIC INDICES			General flea index	Per cent <i>cheopis</i> (whole town)	Total deaths from plague per mille, 1902-1929	Number of years when plague deaths exceeded one per mille, 1902-1929.
			<i>astha</i>	<i>braziliensis</i>	<i>cheopis</i>				
Harpanahalli	7	December	0.02	0.04	6.0	6.1	93	127	14
Tirupattur *	16	April	0.6	nil	1.8	2.4	81	164	9
Berhampore	32	September	0.9	"	3.6	4.6	79	0	0
Hospet	18	December	1.3	,	4.6	5.9	76	197	19
Madanapalli	8	November	3.3	"	6.7	10.0	67	2	1
Combatores	65	January	0.5	1.6	4.0	6.1	64	102	14
Vizagapatnam	44	October	1.5	nil	3.4	5.0	62	11	2
Calicut *	82	February	1.5	0.4	3.5	5.4	59	9	2
Salem	52	February	1.7	nil	2.0	3.7	57	162	12
Bellary	40	October	2.5	"	3.4	5.9	57	163	12
Mangalore	54	March	1.3	1.1	3.3	5.7	57	70	16
Ootacamund	19	May	0.5	0.6	2.1	3.6	49	30	5
Tuticorin	44	October	3.3	nil	1.9	5.2	36	5	1
Guntakal	12	January	2.8	"	1.4	4.2	33	?	?
Dindigul	30	January	1.8		2.4	7.2	33	17	1

	5	September	1 2	8 5	3 8	13 6	25	121	16
Hosur *	50	April	3 1	nil	1 1	4 2	22	24	5
Vellore	18	October	3 7	"	0 9	4 6	21	5	1
Chittoor	16	November	3 8	"	1 0	5 0	20	61	6
Pernakulam *	35	April	2 7	"	0 8	3 4	20		
Nellore	54	September	2 8	"	0 5	3 3	15		
Tinnevely	138	February	3 7	"	0 6	4 3	15	6	1
Madurai	526	July	2 5	"	0 15				
Madras		December	6 1	"	0 7				
Trichinopoly	120	January	6 0	"	0 1	6 1	0 2		
Trumalai	?	May	4 0	"	0	4 0			
Yercaud	?	March	1 4	5 5	0	6 9		?	2
Negapatam	54	June	3 6	0	0	5 8		2	1
		January	5 8	0	0				
Tanjore	60	April	3 3	0	0	3 3			
Trupati	17	May	3 5	0	0	3 5			
Cuddapah	19	September	4 1	0	0	4 1		9	3
Prodattur	16	October	5 1	0	0	5 1			
Cochin *	20	January	1 7	0 4	0	2 1			
Bezwada	44	November	2 4	nil	0 4	2 4		1	1

* Rat epizootic at the time of survey

plague, 1902 to 1929, in towns surveyed

1916.	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	Total
113	8	55	28	3	388	3	3	1	1	1	18	55	1	11	952
472	18	698	22	1	1	1	311	53	1	1	111	87	5	27	3,550
127	573	325	185	32	891	122	810	111	8	1	11	135	17	6,875	505
752	567	733	312	1	279	21	71	15	15	1	29	37	155	753	8,419
13	1	1,071	51	1	1	1	1	1	1	1	1	2	87	0 537	7
116	124	213	94	11	4	29	6	11	5	31	6			3,781	231
34	8	18				10	3	1	1	221		1		605	1
31	28	17	22	5	4	1	1	2	4	2	33	1	0	2	512
82	182	39	2	9	2	1	14	1	19	25	22			1	1,197
82					2									70	984
		1	1		141	1	1	1	2	418					1
		5	22		230				1						2
		11	5	14	8	3	1	1	1	1					198
	2														3
															8
															110
															9
															175
															1
															2
															7
															1
															198
															53

J, MR

TABLE
Total yearly deaths from

[illegible]

Yercaud, a hill station in the Shevaroy Hills, 4,500 feet high and so with a climate favourable for plague, is only 11 miles from the endemic plague centre of Salem through which nearly all its traffic passes, despite which it has had only two small epidemics of plague with 17 deaths—it had no *cheopis*, only *astra* and *brazilensis*

Tirumalai, 2,800 feet with a climate suitable for *cheopis*, is exposed to infection because it is visited by many pilgrims. It has had no plague. Only *astra* were found.

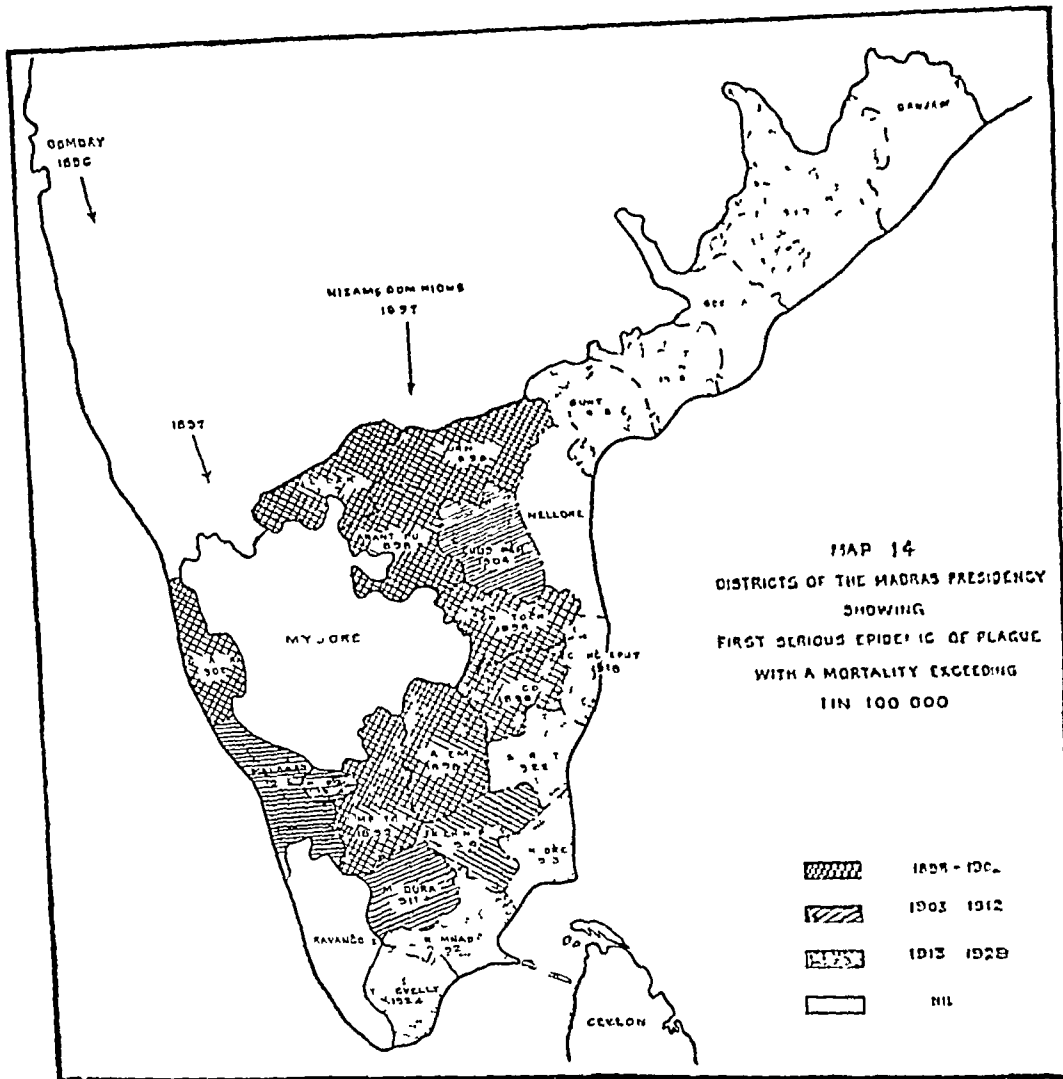
Therefore we conclude that the association between *cheopis* prevalence and plague is probably not wholly due to their both being correlated with climate. Before leaving this map, note how the areas both of greatest plague and of greatest *cheopis* prevalence cluster round the Mysore plateau. This is associated not only with *cheopis* prevalence in this region, but also with frequent importations of infection from Mysore state where plague is practically endemic. Other areas from which plague is imported are the Bombay Deccan, the Nizam's dominions and from the more heavily infected districts of the Presidency itself. The influence of both these factors is well shown by Map 14 giving the years in which plague mortality in districts first exceeded 1 per 100,000 of population (2).

From Bombay in 1896, through the southern parts of the Bombay Presidency and Nizam's dominions in 1897, plague reached the Madras Presidency in 1898 with severe epidemics in some districts. Districts so infected in the first 5 years, 1898—1907, are cross hatched. They are seen to cluster round Mysore. Districts seriously infected in the next 10 years are single hatched, and those similarly infected in the next 17 years are dotted. Districts practically free from plague, i.e., with a mortality less than 1 in 100,000 in any year, are left blank. In interpreting the map two points must be remembered. First, that these are figures for *districts* whereas our own figures are for towns and adjacent villages. Second, that the map *does not represent the time of the first infection*, but only of the first epidemic yielding a mortality exceeding one death per 100,000 for the district. Thus in the first year 1898, plague was imported into 96 different places in 11 districts, but established itself in only 20 centres in 5 districts—Bellary, etc. This emphasizes that earliness of infection on the map shows not only early infection, but also the existence of conditions suitable for epidemics. It is very evident that the map shows a general correspondence between the proportion of *cheopis* previously described in the surveyed towns of a district and the earliness of serious epidemics in the district.

The Chart giving the district mortality curves per 100,000 per year shows that Bellary District is the most affected by plague, and that next come Coimbatore and Salem districts. The heavy intensity in the first two areas is probably associated

with the *cotton trade* and the facilities it gives to the import of fleas. These figures for districts remind us that we need more information on rural conditions.*

MAP 14



The association of *astia* with plague.

We have already noted in Map 13 and Table VII that, in several places where *cheopsis* was probably absent and *astia* or *astia* and *braziliensis* abundant plague has either not occurred at all or has caused only a few mild epidemics. All places surveyed in which *astia* was by far the predominant flea and in which there has been some plague, however little, are noted in Table IX. It is very evident that in

* This chart and Map 14 have been prepared by us from the information given by various diagrams and maps in a Geographical Survey of Plague in the Madras Presidency by Lieut Colonel A J H Russell, I M S, Director of Public Health, Madras

all these places not only have the epidemics been *very few*, and *very small*, but what is far more important, *they have not carried over from one season to another*. The evidence is so definite that there is no need to labour the point, that, places where practically only *astia* exists, are not likely to get severe and recurrent epidemics. It may again be objected, that this is not because the fleas are practically only *astia*, but because the climatic conditions are not favourable to plague transmission. But this is not the case, the climate during the North-East Monsoon and during the cold weather is quite suitable as shown by the following figures for the mean monthly 8 A M temperature in Madras from November to February in the last 3 years

Madras Mean monthly 8 A M temperature and relative humidity

(Average for three years, 1927-29)

	November	December	January	February
Temperature	78.2	76.0	74.7	76.5
Relative humidity	80.6	82.6	86.0	82.3

Then there is the positive evidence of the suitability of the *astia* zone for plague in the moderately serious epidemic of plague in places like Vellore and Tuticorin, which, while being in or near the *astia* climatic zone with 100 per cent *astia* in purely residential areas, yet have many *cheopis* in their bazaar areas—22 per cent and 30 per cent respectively. Excluding plague deaths below 10 a year, Vellore has had ten plague epidemics yielding 24 deaths per thousand of population to date. Tuticorin has had one epidemic recently in 1924 yielding 5 deaths per thousand. These are much higher death rates than in the towns mentioned in Table IX where only *astia* was at work and though perhaps climate has played a part in promoting the difference for these towns are on the borders of the *astia* zone, yet it is probable that the higher plague rates are due mainly to the presence of *cheopis*.*

The association of *X. braziliensis* and *Ceratophylus nilgeriensis* with plague.

Plague occurred in Yercaud in 1911 and 1921 with 17 deaths. Here the fleas were 79 per cent *braziliensis* and 21 per cent *astia*, so it is probable that the two epidemics were caused by *braziliensis* as well as by *astia* which we know can convey plague.

In Hosur, which suffers severely from plague, *braziliensis* was the commonest flea forming 63 per cent of all the fleas and having the high specific index of 8.5, so

* The evidence given above in favour of plague epidemics not being carried over the off season by *X. astia* acting alone, while they are so carried over when *X. cheopis* is present, suggests that plague epidemics are carried over by the persistence of infection in rat fleas rather than in rats.

it is probable that it plays some part in plague transmission, but this is not certain since the *cheops* index was as high as 3.8. Here a rat was seen in the act of dying with plague. It yielded 32 *braziliensis* and only 1 *cheops* which strongly suggests that *braziliensis* had probably carried the disease.

As noted in Table IX, in Cochin plague has occurred only twice—in one part, Mattancherry, the last occasion being as recent as 1928. Very few human cases then occurred—only 14, but many rat-falls. In the residential areas of Mattancherry where the rat-falls were few the indices were 1.8 for *astia* and 0.2 for *braziliensis*. In the bazaars where there were very many rat-falls the specific indices for *astia* and *braziliensis* were practically the same—1.3 and 1.2. This suggests that *braziliensis* is at least as efficient a vector as *astia*. The fact of the rat-falls officially recorded in Mattancherry—660—being out of all proportion to the few human cases, rather suggests that the species responsible (*astia* or *braziliensis* or both), while freely carrying plague to rats, do not readily convey plague to man,

TABLE IX
Astia plague epidemics

Town	Population in thousands	Flea and plague data
Cuddapah	20	100 per cent <i>astia</i> Plague—1903 and 1912 1918 and 1919 No records 93 deaths
Bezwada	44	99 per cent <i>astia</i> , 100 per cent in residences Plague—1917 and 1918 53 cases Importation common
Madras	526	94 per cent <i>astia</i> , 100 per cent in residences Plague—1905 60 deaths, all in Kassimodu where now only <i>astia</i> with a high index 6.1 1918 31 deaths in main town where only <i>astia</i>
Negapatam	54	100 per cent <i>astia</i> Plague—1913 and 1914 140 deaths, infection probably from Rangoon
Cochin	21	83 per cent <i>astia</i> , 17 per cent <i>braziliensis</i> , no <i>cheops</i> , <i>astia</i> 98 per cent in residences Plague—1919 and 1925 in Mattancherry, 20 and 11 deaths respectively
Bellary Cantonment	?	100 per cent <i>astia</i> Plague—Only 7 cases of plague in the last 16 years despite nearness of Bellary where plague is very preva- lent
Yercaud	?	21 per cent <i>astia</i> , 79 per cent <i>braziliensis</i> Plague—1919 and 1921 with 7 deaths

but as previously noted, the indices were low which alone may have prevented transmission of plague to man

As regards *Ceratophylus nilgiriensis*, as noted in the report for Ootacamund, it is probably associated with *cheopis* in the transmission of plague in the Nilgiris

The flea species factor in plague

We have demonstrated a marked association between *cheopis* distribution and climate, and a similar marked association between *cheopis* distribution and plague. We have given some evidence that this latter association is not due merely to an association between climate and plague apart from the flea species factor by showing that in the absence of *cheopis* in some places favourable climatically to plague there has been none or very little human plague, despite the occasional presence of plague infection as shown both by human and rat plague. The evidence that the association of plague with *cheopis* is not wholly due to a common association with climate is very much strengthened if we consider the *cool weather* climates of the typical *astria* zone in the south-east plain. Here, after the onset of the North-East Monsoon in October, for the next 4 months we have a climate eminently suitable for plague propagation, but it does not occur despite flea indices that are quite high enough for plague epidemics did they refer to *cheopis*, e.g., Madras residences gave an *astria* index of 5.8 in the cold weather. When it is borne in mind that in these places the rats are highly susceptible to plague, far more so than in *cheopis* areas, and when further it is remembered that many of these places are *important trade centres* receiving plague infection both from outside ports and from the western hinterland, then we think that the evidence is good enough to allow us to conclude that in South India *the flea species factor is of the first importance in the spread of plague under natural conditions* and that *X cheopis* is undoubtedly the chief vector, far more efficient than the common indigenous flea, *X astria*, and possibly more efficient than *X braziliensis*, the plateau flea, but there is not enough evidence for this second comparison. Webster(3) and Goyle(4) have clearly shown in their important experiments that *astria* freely conveys plague. But we must remember that the transmission of plague in laboratory experiments is apt to be deceptive as to the part played by each species in nature, unless care be taken to imitate natural conditions as far as possible, with due regard to the general environment, facility of the contact of fleas with rats, and the *flea density*. As regards this last point, we understand that Webster in his recent work at Bombay concludes that *astria* needs a higher index to convey plague than does *cheopis*. Finally, it must be remembered that, unlike epidemiological evidence, laboratory experiments do not give conclusions for the conveyance of plague to *man*.

So far as South India is concerned our survey confirms the theory of Hust(5) which was supported by Ciagg(6*a* and *b*) that plague in India is mainly caused by *cheopis*.

We are so struck both with the far greater efficiency of *cheopis* as a vector in nature and with the evidence that the species is of comparatively recent introduction, that we suggest that the explanation of the recent history of plague in India from 1896 onwards and why this is different from previously recorded epidemics which rapidly died out, is that whereas previous epidemics occurred in the absence of *cheopis*, the 1896 infection occurred when *cheopis* was fairly widespread throughout India as a whole. We suggest that the dissemination of *cheopis* occurred after the extension of human intercourse and trade (particularly the cotton trade) with Egypt following the opening of the Suez Canal in 1869. Probably the process of *cheopis* importation began even before this. Thus, Choksy (7) suggests that the actual infection was imported into Guzarat from Egypt in 1815 with cotton and caused the epidemic of that year. Whether that was so or not it is apparent that the Bombay Presidency was importing Egyptian cotton at that time. Whereas all previous epidemics seem to have behaved like typical 'austrian epidemics' in being localized and in not carrying over from one year to another the epidemic since 1896, as we in India know to our cost, has behaved very differently.

Conclusions

(1) Local knowledge in survey work is essential because the factors of importation and environment vary in different parts of the same town.

(2) Locality suitability appears to be able to make up to some extent for climatic defects.

(3) *X. astra* is an indigenous flea of South India.

(4) *X. braziliensis* seems established in the Mysore Plateau and adjacent areas.

(5) *X. cheopis* appears to have been comparatively recently introduced and is still spreading, or attempting to spread, into fresh areas chiefly by the help of the cotton and grain trades.

(6) The area least suited to *cheopis* is the central and southern part of the East Coast plain, and that these limits are associated with the effect of climate in prolonging the off-season here where the South-West Monsoon is very weak.

(7) The occurrence and severity of plague epidemics are associated with the number of *cheopis* present and that this relation is mainly direct and not just because of the common association of plague and *cheopis* with climate.

(8) However else plague may be associated with climate, its chief association appears to be through the effect of climate on flea species.

(9) *X. astra* acting as a vector in nature without *cheopis* in South India has produced very few and very small epidemics which did not carry over the off-season.

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SOME SOURCES OF VITAMIN C IN INDIA

THE ANTISCORBUTIC VALUES OF THE FRUITS

Part II.—*Contd.*

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[Received for publication, April 18, 1931]

THIS investigation forms another chapter to a previous inquiry published in the *Indian Medical Gazette* (Vol LXIV, No 2, February 1929).

The nature of this and the previous inquiry may be classified as a 'routine phase of a physiological research but as no enquiry of the kind has been carried out in India, the publication of these results may be of some interest to the dietitians. A vast amount of work on the subject has been done in Europe and America that the conclusions arrived at there are not applicable to India will be evident from the perusal of the following report. It will be evident that vitamin C content of the various fruits differ according to the variety of the article and the environment in which they have been grown. The protective values of the identical fruits, e.g., oranges do not correspond to the values arrived at by English workers. These facts have been proved by McCarrison(1), in regard to nutritive and vitamin values of millet and wheat, and recently Bracewell, Hoyle and Zilve(2) have pointed out the marked differences in vitamin C content of the different types of apples. There is no doubt that vitamin C, as compared to other vitamins, seems to belong to a different category regarding its distribution, it may be present in abundance in one or two closely related varieties of fruits and absent from another, notwithstanding the fact that they are growing side by side.

The fruits we have investigated were bought at Maymyo (Burma) being brought to the market from the surrounding villages. They were ripe and wholesome but by no means of the 'best' variety from cost point of view, as our effort was to confine this inquiry to the articles, which the 'man in the street' could afford for his domestic consumption.

It is quite possible that the superior quality of the fruits if used in these experiments might have proved better in their antiscorbutic properties, e.g., the Kandhar pomegranates and Kulu pears, but they are not available in the average Indian town and their prohibitive cost prevents their use by the average Indian populace.

The details of the experimental work are given at the end of the paper as the scrutiny of these is essential to judge the methods which have been employed and the inferences which have been drawn.

Guinea-pigs, as usual, were used for assaying the antiscorbutic value.

A modified basal diet containing chick-pea or gram (Cicer arietinum)

The routine basal diet used for producing scurvy among guinea-pigs consists of bran and oats *ad lib* with 60 c.c. of autoclaved milk. The method followed is similar to that of the Norwegian workers except the addition of heated milk which was introduced by Chick and Hume (*Jour. R. A. M. C.*, August, 1917) to eliminate the complications caused by inanition and starvation. We have made a very convenient modification by omitting the milk and substituting 30 per cent of crushed gram or gram meal in the usual basal diet. By this addition we have not been able to detect any appreciable change in the special symptoms of the disease or its time of onset. The animals on this diet have kept up their general condition fairly well as compared with those having milk in addition. In this connection we have looked up the previous paper of this series as well as compared the weight of the control animals of other workers and find that the diet works quite satisfactorily. The nutritive value of gram (albuminoids 19 per cent, starch 53 per cent and oil 4.5 per cent) is too well known to be discussed here, but it may be mentioned that crushed gram or gram meal has no antiscorbutic value, even though the material is given after soaking overnight (*see* Group I, controls). We recommend the use of gram meal instead of the crushed gram when the animal has developed the 'face ache,' or other symptoms of scurvy, as chewing of the gram itself is rather painful.

The juice or pulp of the fruit to be tested was mixed with a small quantity of the basal diet and served out to the guinea-pigs every morning. After the animals had consumed this portion a liberal quantity of the basal diet was given. In the case of orange and pomegranate juice, artificial feeding was resorted to by means of a pipette. This was carried out to make doubly sure that the animals got the juice, as the results of a previous experiment where doubted, the orange having such a good reputation as an antiscorbutic.

The experimental animals were divided into the following groups

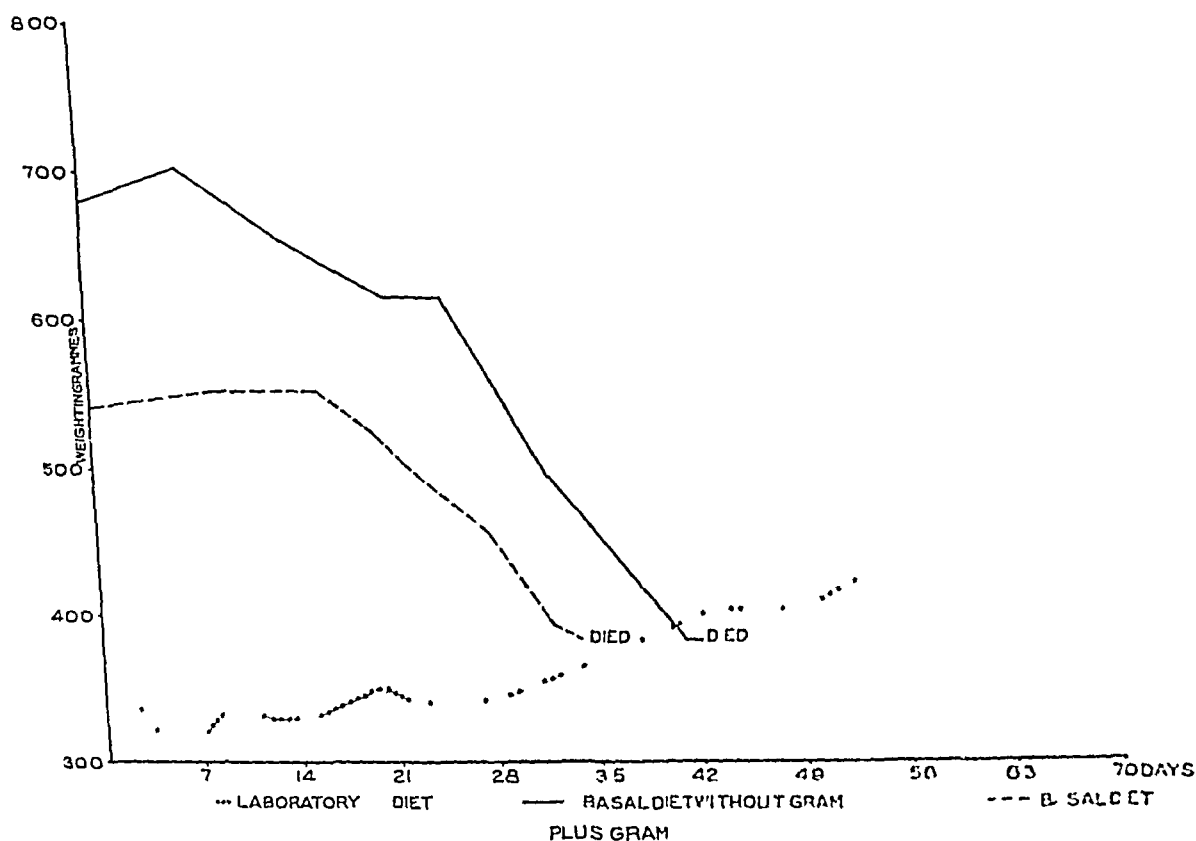
Group 1 Controls

(a) One animal on laboratory diet consisting of lucern, crushed gram, lucern and oats

(b) Two animals, one on the usual basal diet of bran and crushed oat and the other on crushed gram in addition

GROUP 1

Controls



Group 2 Pumelos (*Citrus decumana*)

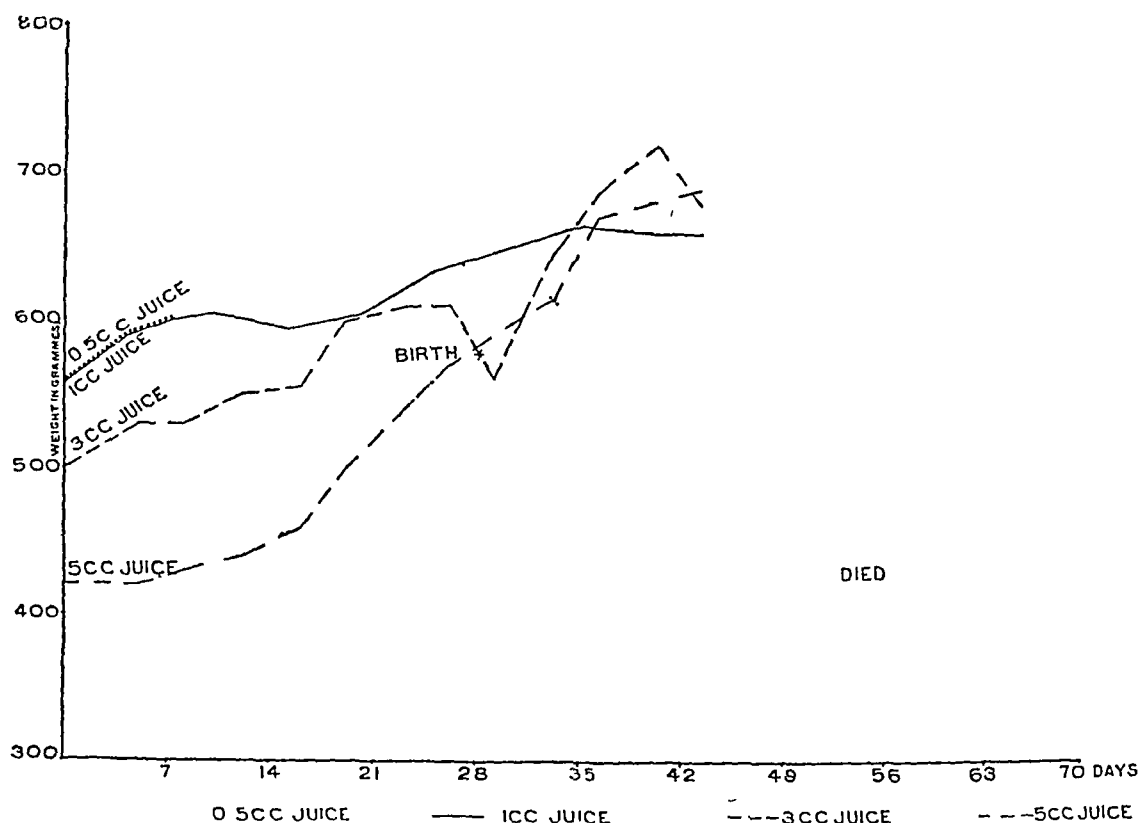
(The fruit is globose and orange-like but much larger, is pale yellow in colour with very thick peel. The pulp is yellow or crimson and contains pleasantly acid juice) Four animals were put on the basal diet with $\frac{1}{2}$, 1, 3 and 5 c.c. of pumelo juice in addition

Group 3 Sweet limes (*Citrus medica* var *limetta*)

(The fruit is oblong with thin yellow rind, the pulp is yellowish white with pleasant sweet taste) Three animals on basal diet with 1, 3 and 5 c c of sweet lime juice in addition

GROUP 2

Pumelos



The line with the word 'died' should stop between 42 and 49

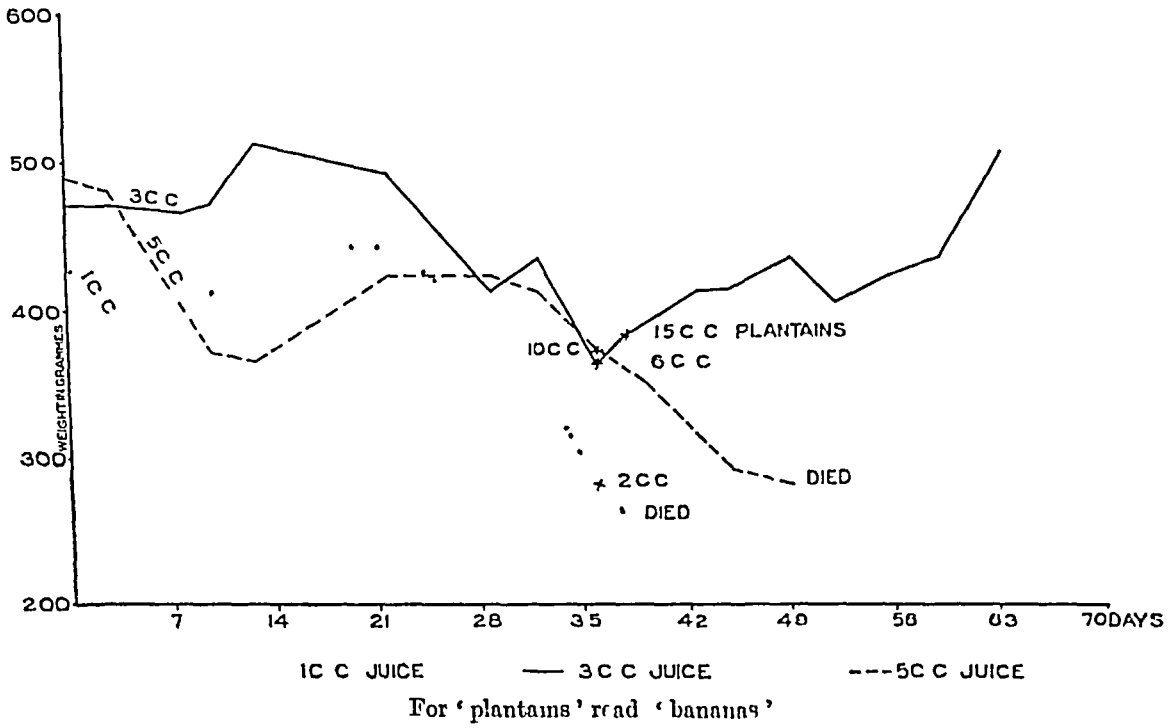
Group 4 Oranges

(The variety resembles 'Nagpur' oranges sold all over India except that it is smaller The rind is very loose round the deliciously flavoured pulp) Three animals on basal diet with 3, 5 and 10 c c of juice in addition

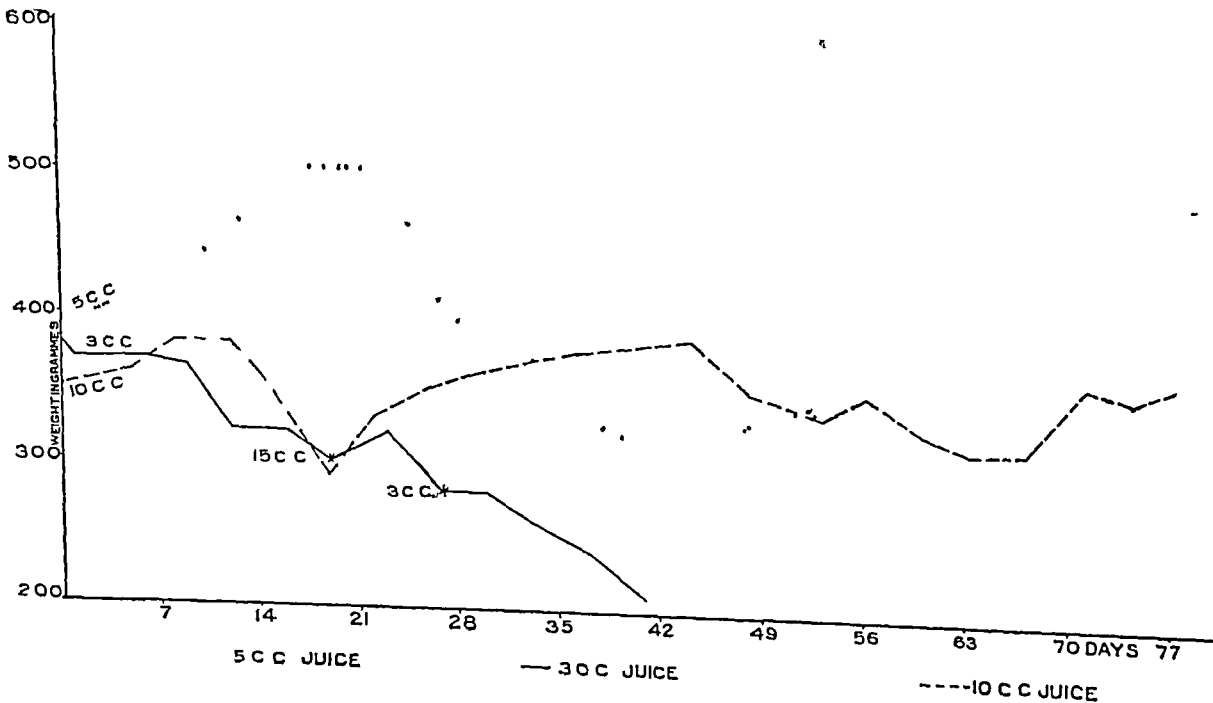
Group 5 Pine-apples (*Bromelia ananas*)

(It is a compound conical fruit borne on a short stalk with succulent fleshy and delicious pulp) Three animals on basal diet with 1, 3 and 7 gm of peeled pine-apple chunks (not juice) in addition

GROUP 3.
Sweet limes.



GROUP 4
Oranges

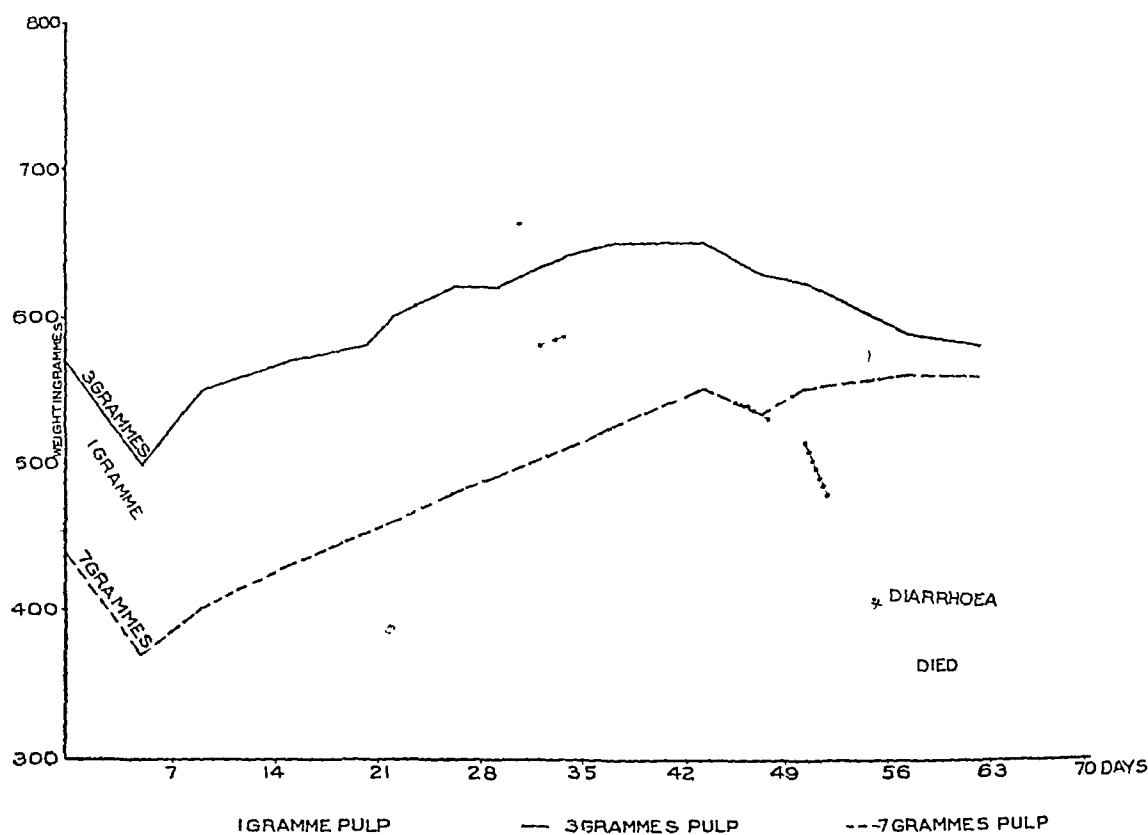


Group 6 Bananas (*Musa paradisiaca*)

(It must not be confused with plantain which is unedible unless cooked. The fruit is about 6 inches in length, yellow in colour and has 3 or 4 longitudinal ridges marked on the rind.) Three animals on basal diet with 5, 10 and 15 gm of peeled bananas in addition

GROUP 5

Pine-apples



The line with 'diarrhoea' and 'died' is marked a little too far. It should end with 'died' between 49 and 56

Group 7 Pomegranates (*Punica granatum*)

(The fruit resembles the Kandhar variety except that it is smaller and the pulp round the seeds is whitish in colour and has rather an astringent subacid taste.) Four animals on basal diet with 1, 3, 5 and 7 c c of juice in addition

Group 8 Pears (*Pyrus communis*)

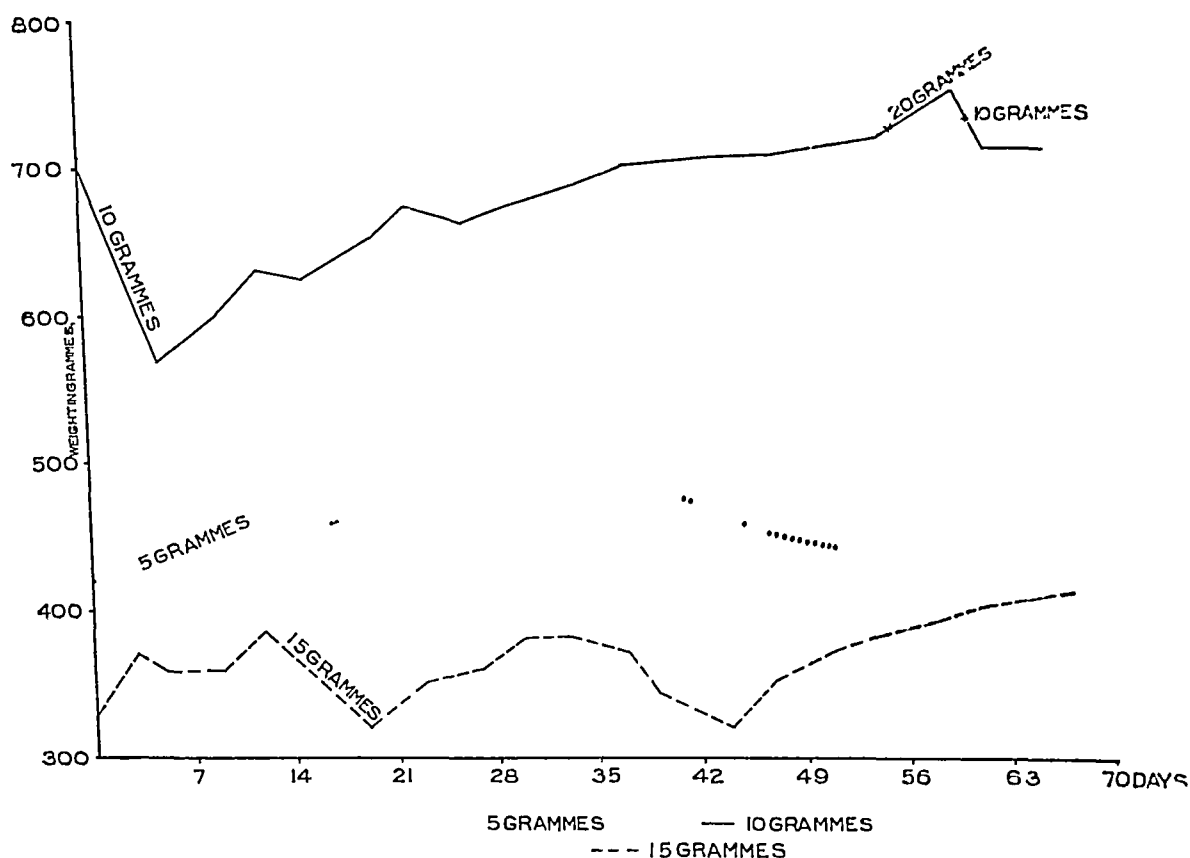
(It resembles the pears sold in India but they are of a miniature size and not so pleasant in taste.) Three animals on basal diet with 3, 5 and 7 gm of peeled pears in addition

In the case of Group 1, controls, the animal on laboratory diet increased steadily in weight until termination of the experiment after which no observations were made

The animal on basal diet without gram died of scurvy on the 43rd day having lost about 300 gm of its initial weight

GROUP 6

Yellow table bananas



The animal on the basal diet, with gram included, died of scurvy on the 34th day having lost 160 gm of its initial weight

In Group 2, pumelos, except for the animal on $\frac{1}{2}$ c c of pumelo juice which died of scurvy on the 52nd day of the experiment, the rest survived, steadily gaining in weight almost throughout the experiment

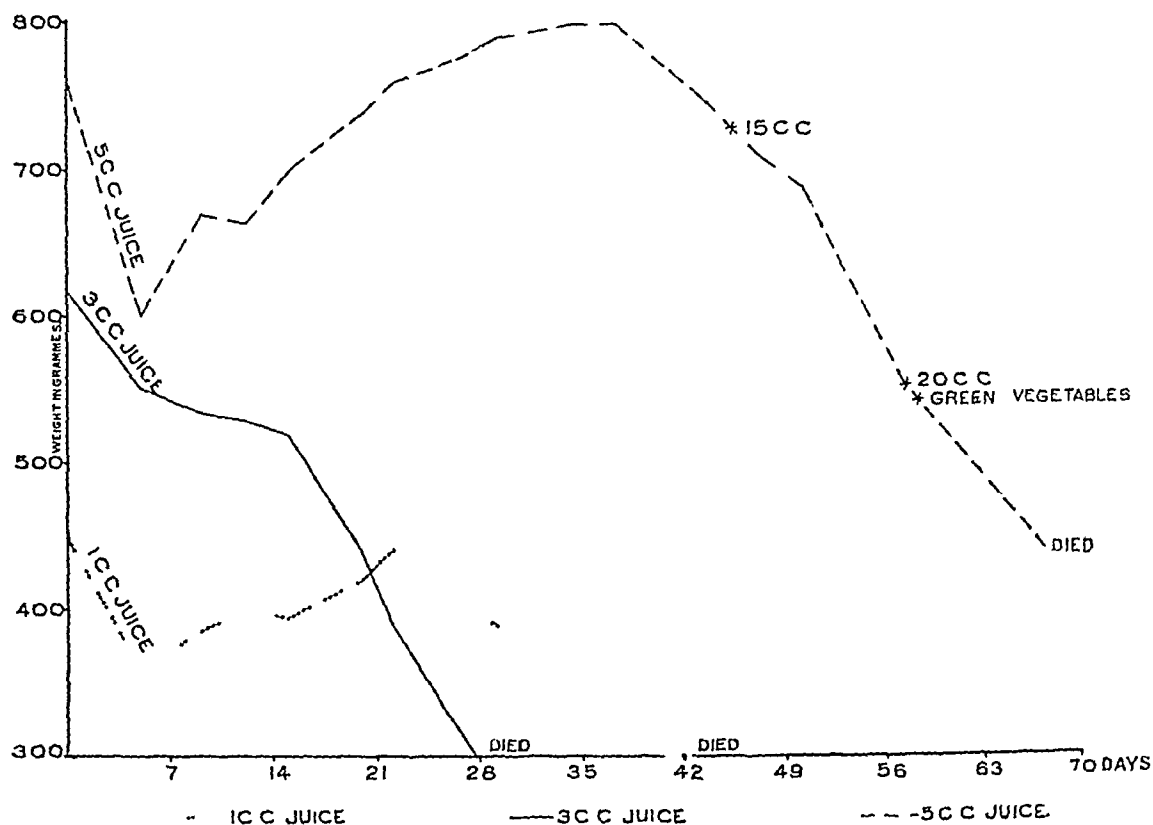
In Group 3, sweet limes, all the animals lost weight and ultimately died. The post-mortem revealed the usual picture of scurvy. The experiment would have

been repeated with larger quantities of the juice if the fruit had not gone out of season

In Group 4, oranges, the animal on 3 c c of the juice gradually decreased in weight until the termination of the experiment, i e , 40th day, when its weight had fallen to a quarter of the initial weight

The animal on 5 c c of the juice steadily rose in weight until the 23rd day when its weight commenced to fall till the termination of the experiment, i e , 64th day, when it showed a weight of 280 gm as against its initial weight of 390 gm

GROUP 7 Pomegranates



(For 7 c c see Appendix, Group 7)

The upper line with the word 'died' should end above 63

The animal on 10 c c behaved similarly to that on 5 c c at first, but then came down to its initial weight remaining thence steadily at the same weight throughout the experiment

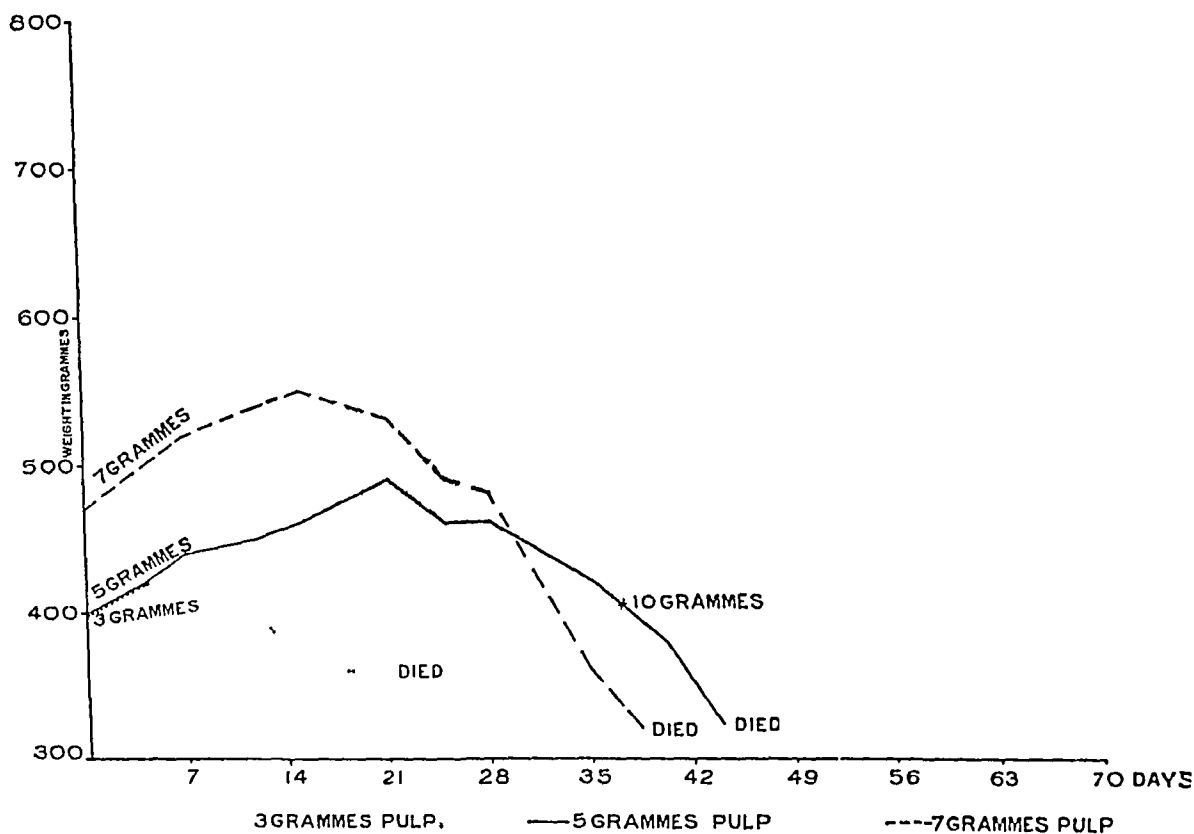
It would have been desirable to continue the experiment in case of the guinea-pigs on 3 and 5 c c of the juice, but unfortunately the fruit had gone out of season

however we have no doubt that the animals would have died of scurvy as the weight was on the decrease day by day

In Group 5, pine-apples, the animal on a gm increased in weight until the 38th day when it began to decrease in weight, developed diarrhoea and died on the 55th day No signs of scurvy could be found at the post-mortem

GROUP 8

Pears



The two upper lines with the word 'died' should end above 35 and 42 respectively

The remaining 2 animals gradually increased in weight and at the termination of experiment had gained 15 gm and 120 gm respectively as compared to the initial weights

Group 6, bananas, all the three animals showed an increase in weight and were in good condition at the end of the experiment

In Group 7, pomegranates, it was found that 7 c c of the juice were insufficient to keep the animals alive To test the therapeutic effect of the juice, the dose for the animal on 5 c c which was losing weight rapidly, was increased to 15 c c on

the 44th day but to no effect. The experiment was repeated with an initial dose of 3 c c of the juice increased to 12 c c after a fortnight and to 20 c c after a month, but no improvement resulted and the animal died on the 36th day.

In Group 8, pears, all the animals died although the amount of fruit pulp was increased to 10 gm on the 37th day in the case of the animal on 5 gm of the fruit.

STATEMENT OF RESULTS

The basal diet —The basal diet with crushed gram worked very satisfactorily as the animals thrived well on it and did not develop any intestinal disturbances, only one animal having died of diarrhoea out of the total 25 experimented upon.

Pumelos —The fruit seems to be superior in its antiscorbutic values to orange and lemon, requiring 1 c c of the juice to prevent the development of scurvy in guinea-pigs.

Sweet limes —These seem to be rather poor in their vitamin C content, as 5 c c of the juice were insufficient to prevent the animal from developing scurvy.

Oranges —These do not compare favourably with the antiscorbutic value arrived at by Western workers, this undoubtedly being due to the 'racial' difference in the species which is more allied to 'Nagpur' variety than to the 'Malta' orange available in England and America. The guinea-pig on 10 c c of the juice kept up the antiscorbutic balance but the gradual decrease of weight in the animals on 3 and 5 c c of the juice would have terminated in death from scurvy if the experiment could have been continued.

Pine-apples —This fruit was found to be the second best of the series, the best being pumelo. It is superior to lemon and orange juice, 3 gm of the fruit pulp being sufficient to keep off scurvy in guinea-pigs. The fruit pulp of the fresh fruit contains 60 per cent of the juice by weight so that a little over 2 c c would have been adequate for the purpose.

Bananas —The antiscorbutic value arrived at came, rather, as a surprise. No quantities smaller than 5 gm were tried but this proved to be quite adequate for the prevention of scurvy. The therapeutic effect of this fruit is evident from the graph of the sweet lime series. When the animal receiving 5 c c of juice had developed symptoms of scurvy and was losing weight, the substitution of banana pulp cured and prevented the disease as well as enabled the animal to increase in weight steadily.

Prescott has described the banana as a 'high power fuel food, worthy of being considered a staple article of diet'. The composition of banana and potato shows an interesting similarity regarding total carbohydrates and minerals, having practically the same caloric value but the former has the advantage over the latter as it can be eaten raw thus ensuring the administration of thermolabile vitamin C. The poor antiscorbutic value found by Western workers is most probably due to the long interval between the collection and consumption of this fruit.

Pomegranates —These were very poor in vitamin C content, even 20 c c. proving useless for the purpose

Pears —Up to 10 gm were no good for antiscorbutic purposes.

EVALUATION OF VITAMIN C CONTENT

For efficient use of vegetables and fruits as antiscorbutics, it is important to be aware of the amount of the vitamin present in the various food-stuffs. In spite of the great strides which vitamin research has made in the last few years, no satisfactory method so far has been fixed to indicate quantitatively the presence of this vitamin. The relative values are indicated usually by one or more plus signs which gives a very rough idea of the presence of the antiscorbutic element. The results, arrived at by Hess(3) wherein the average value of 1 gm of lemon juice is taken as 100, is undoubtedly more satisfactory but one can not calculate from the table given the dosage required for an adult human being. The principle of pharmacology, to arrive at adult dosage, by means of animal experiment, has not been applied to the vitamins as the requirements of various age groups vary so much, and moreover, under normal conditions, the vitamin requirements are amply covered by the ordinary diet. The calculation may, however, serve the purpose of expressing quantitatively the extent of the antiscorbutic element, giving a better idea than one or more plus signs.

To arrive approximately at the result is not difficult, if we keep in mind the dosage required to protect a guinea-pig from scurvy and compare it with the amount required for a human being. In case of lemon juice the amounts are 3 c c and 30 c c respectively, i e., the ratio is 1 : 10, this ratio has been confirmed in the case of orange juice and some vegetable juices. Taking this ratio as the basic principle, the value of the fruit and vegetables investigated by us may be expressed as follows —

Names	Amount required for adult human being, the article in question being the only source of vitamin C
<i>Fruits</i>	
Bananas	50 gm
Lime juice	50 c c
Orange juice	100 c c
Pears	Very poor, 70 gm merely delay onset of scurvy

Names	Amount required for adult human being, the article in question being the only source of vitamin C
<i>Fruits—concd</i>	
Pine apples .	30 gm of pulp
Pomegranate juice .	Very poor, up to 200 c c inadequate.
Pumelo „ .	10 c c
Sweet lime „	Poor, 50 c c inadequate
<i>Vegetables</i>	
Melon pumpkin	100 gm.
Pumpkin	100 gm
Vegetable marrow	150 gm

We would like to point again that the above table is not meant to calculate the dosage for various ages on the same lines as the dosage of drugs in the *Materia Medica*, for a child of 8 to 10 kilos would require about half the dose of an adult. The value is also dependent upon a variety of factors, e g , the species of fruit, and the influences of the environment in which it is grown , hence too rigid an adherence to the above values is not possible. The values have been tabulated taking the human factor into consideration instead of that of the guinea-pig, merely to suggest a different, perhaps a more satisfactory method, of expressing the vitamin C of the various articles of food.

In the end we would like to record our thanks to the Hon'ble Major-General J W D Megaw, C I E , M B , I M S , who encouraged us to take up the work and to Colonel R McCarrison, C I E , M D , F R C P , I M S , from whom we received very valuable suggestions.

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- | | |
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| (2) BRACEWELL, HOYLE and ZILVE | The antiscorvy vitamin in apples <i>Medical Research Council Special Report Series</i> , No 146 |
| (3) HESS (1929) | .. <i>Brit Med Jour</i> , July 31, p 154 |

APPENDIX

GROUP 1. CONTROLS

Date	Weight of animal on ordinary laboratory diet in grammes	REMARKS	Date	Weight of animal on usual diet without crushed gram in grammes	REMARKS	Date	Weight of animal on usual diet with crushed gram in grammes
19-7-30	370		18-5-30	680		12-6-30	510
24-7-30	300		22-5-30	690		21-6-30	550
28-7-30	340		25-5-30	700		28-6-30	550
31-7-30	330		1-6-30	650		10-7-30	450
3-8-30	330		8-6-30	610		11-7-30	390
8-8-30	350		12-6-30	610		16-7-30	380
						Died on 16th July, 1930 Post mortem showed hemorrhages, subserous and around both knee joints	
10-8-30	340		19-6-30	490			
14-8-30	340		28-6-30	380			
17-8-30	345		29-6-30	380	Died Post-mortem showed hemorrhages, subcutaneous, subserous, and around joints especially the knee joints		
22-8-30	365						
25-8-30	375						
31-8-30	400						
4-9-30	400						
7-9-30	400						
15-9-30	440						
20-9-30	420						
25-9-30	430	Released in good condition					

GROUP 2 PUMELOS

Date	Weight of animal on 1 c c of juice in grammes	REMARKS	Weight of animal on 3 c c of juice in grammes	REMARKS	Weight of animal on 5 c c of juice in grammes	REMARKS
13-4-30	560		500		420	
17-4-30	590		530		420	
20-4-30	600		530		430	
23-4-30	620					
24-4-30			550		440	
28-4-30	595		555		460	
1-5-30	605		600		500	
5-5-30			610		540	
6-5-30	635					
8-5-30			610		570	
11-5-30	650		568		590	
15-5-30			645		620	
16-5-30	665					
18-5-30			690		670	
21-5-30	660					
22-5-30			720		680	
24-5-30	660					
25-5-30		Released in good condition	680	Released in good condition	690	28-5-30 Released in good condition

GROUP 2 PUMELoS.

Date.	Weight of animal on $\frac{1}{2}$ o c of juice in grammes	RE MARKS
24-7-30	560	
28-7-30	590	
31-7-30	600	
3-8-30	620	
8-8-30	625	
10-8-30	635	
14-8-30	640	
17-8-30	650	
22-8-30	630	
25-8-30	620	
31-8-30	570	
4-9-30	525	
7-9-30	490	
14-9 0		Died Post-mortem Hæmor- rhages seen subserous and around both knee joints Costocondral junctions thick- ened and hæmorrhagic

GROUP 3 SWEET LIMES

Date	Weight of animal on 1 cc of juice in grammes	REMARKS	Weight of animal on 3 cc of juice in grammes	REMARKS	Weight of animal on 5 cc of juice in grammes	REMARKS
11-9-30	430		470		490	
14-9-30	390		470		480	
19-9-30	420		465		400	
21-9-30	420		470		370	
24-9-30	440		510		365	
2-10-30	440		490		420	
9-10-30	390		410		420	
12-10-30	550		430		410	
16-10-30	280	Put on 2 cc	360	Put on 6 cc	370	Put on 10 cc
18-10-30		Died Post mortem subserous hæmorrhages, suparenals enlarged and hæmorrhagic				
19-10-30			380	Put on 15 gm of banana pulp	350	
24-10-30			410		310	Hind legs seem painful on movement Put on 20 cc
26-10-30			410		290	
30-10-30			430		280	
2-11-30			400			Died 11130 Post mortem hæmorrhages around joints, subserous hæmorrhages
6-11-30			420			
9-11-30			430			
13-11-30			500			
14-11-30			.	Released in good condition		

GROUP 4 ORANGES

Date	Weight of animal on 3 cc of juice in grammes	REMARKS	Date	Weight of animal on 5 cc of juice in grammes	REMARKS	Date	Weight of animal on 10 cc of juice in grammes	REMARKS
31-12-30	380		6-12-30	390		15-11-30	350	
1-1-31	370		11-12-30	410		20-11-30	360	
5-1-31	370		14-12-30	430		23-11-30	380	
8-1-31	365		18-12-30	450		27-11-30	380	
11-1-31	320		21-12-30	500		29-11-30	360	
15-1-31	320		29-12-30	450		4-12-30	290	
18-1-31	300	Put on 15 cc	2-1-31	400		7-12-30	330	
22-1-31	320		5-1-31	380		11-12-30	350	
26-1-31	280	Put back on 3 cc	8-1-31	355		14-12-30	360	
29-1-31	280		11-1-31	325		18-12-30	370	
1-2-31	260		15-1-31	315		21-12-30	375	
5-2-31	240		18-1-31	300		29-12-30	385	
9-2-31	210	Released in very poor condition	22-1-31	330		2-1-31	350	
			26-1-31	340		7-1-31	335	
			29-1-31	300		10-1-31	350	
			1-2-31	300		14-1-31	325	
			5-2-31	280		17-1-31	315	
			9-2-31	280	Released in poor condition	21-1-31	315	
						25-1-31	360	
						28-1-31	350	
						31-1-31	360	
						4-2-31	360	
						7-2-31	340	Released in good condition

GROUP 5 PINE-APPLES

Date	Weight of animal on 1 gm of pulp in grammes	REMARKS	Weight of animal on 3 gm of pulp in grammes	REMARKS	Weight of animal on 7 gm of pulp in grammes	REMARKS
9-7-30	530		570		440	
24-7-30	450		500		370	
28-7-30	500		550		400	
31-7-30	520		560		415	
3-8-30	530		570		430	
8-8-30	560		580		450	
10-8-30	570		600		460	
14-8-30	750		620		480	
17-8-30	580		620		490	
22-8-30	600		645		510	
25-8-30	600		650		525	
31-8-30	540		650		550	
4-9-30	515		630		535	
7-9-30	450		625		550	
9-9-30		Diarrhœa				
11-9-30		Died Post mortem				
14-9-30		showed signs of scurvy	590		560	
19-9-30		Circular areas of necrosis seen under the subserous coat of the intestine Descending colon distended	585	Released in good condition	560	Released in good condition

GROUP 6 YELLOW TABLE BANANAS

Date	Weight of animal on 5 gm of pulp in grammes	REMARKS	Date	Weight of animal on 10 gm of pulp in grammes	REMARKS	Date	Weight of animal on 15 gm of pulp in grammes	REMARKS
31-7-30	420	Released in good condition	19-7-30	700		21-10-30	330	
3-8-30	420		24-7-30	570		24-10-30	370	
8-8-30	440		28-7-30	600		26-10-30	360	
10-8-30	440		31-7-30	630		30-10-30	360	
14-8-30	450		3-8-30	625		2-11-30	385	
17-8-30	460		8-8-30	650		6-11-30	300	
22-8-30	480		10-8-30	670		9-11-30	320	
25-8-30	495		14-8-30	660		13-11-30	350	
1-9-30	500		17-8-30	670		17-11-30	360	
4-9-30	495		22-8-30	685		20-11-30	380	
7-9-30	480		25-8-30	695		23-11-30	380	
14-9-30	450		31-8-30	700		27-11-30	370	
19-9-30	440		4-9-30	700		29-11-30	345	
			11-9-30	710		4-12-30	320	
			12-9-30		Put on 20 gm	7-12-30	350	
			17-9-30	740		11-12-30	370	
			18-9-30		Put back on 10 gm	14-12-30	380	
			19-9-30	700		18-12-30	390	
			23-9-30	700	Released in good condition	21-12-30	400	
						27-12-30	410	Released in good condition

GROUP 7 POMEGRANATES.

Date	Weight of animal on 1 cc of juice in grammes	REMARKS	Weight of animal on 3 cc of juice in grammes	REMARKS	Weight of animal on 5 cc of juice in grammes	REMARKS
19-7-30	450		620		740	
24-7-30	360		550		600	
28-7-30	385		535		670	
31-7-30	400		530		665	
3-8-30	395		520		700	
8-8-30	420		440		740	
10-8-30	440		390		760	
14-8-30	400		330	Died 15 8 30	775	
17-8-30	390			Hæmorrhages in intestinal subserous coat, costal junctions	790	
22-8-30	355	Movement of hind legs seems limited Started on lucern grass		Suprarenals enlarged and hæmorrhagic	800	
25-8-30	340			Stomach full of hæmorrhagic fluid	800	
30-8-30		Animal seems brighter		Another animal was fed on initial amount of 3 cc, increased to 12 cc after a fortnight and again to 20 cc after a month, but did not survive	750	Put on 15 cc
31-8-30	280	Died Post mortem no evidence of scurvy				
2-9-30	.				710	
4-9-30					690	
7-9-30					555	Put on 20 cc pumelo juice
14-9-30						Put on green vegetables
15-9-30					500	
19-9-30			.			
24-9-30			..	Died of scurvy on the 36th day		Died on 24 9 31 Hæmorrhages seen in serous coat of bladders, stomach and intestine Suprarenals hæmorrhagic

Result of experiment on the animal on 7 cc of the juice in addition to basal diet —

Initial weight	Duration of experiment	Ultimate weight	Condition of animal on termination of experiment
500 gm	37 days	170 gm	Very poor Movements of hind legs painful

GROUP 8 PEARLS

Date	Weight of animal on 3 gm of pulp in grammes	REMARKS	Weight of animal on 5 gm of pulp in grammes	REMARKS	Weight of animal on 7 gm of pulp in grammes	REMARKS
10-8-30	400	Died Post-mortem teeth loose, hæmorrhages around joints in subcutaneous tissues Sciatic nerve shows degeneration	400	Put on 10 gm	170	Died 17-9-30 Post mortem hæmorrhages around joints Hæmaturea Hæmorrhages in subserous coat of bladder and intestine
14-8-30	420		420		500	
17-8-30	420		440		520	
22-8-30	400		450		510	
25-8-30	360		460		550	
31-8-30	360		190		530	
4-9-30			460		490	
7-9-30			460		480	
14-9-30			420		460	
16-9-30						
19-9-30			380			
21-9-30			350			
23-9-30						
				Died Post mortem subserous hæmorrhages of bladder and intestine Hæmorrhages around knee joints		

ADDICTION TO 'POST'—UNLANCED CAPSULES OF *PAPAYER SOMNIFERUM*

Part II.

COMPOSITION OF LANCED AND UNLANCED CAPSULES

BY

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Drug Addiction Series No 12

[Received for publication, April 20, 1931]

IN a previous paper on Addiction to 'Post'—unlanced capsules of *Papaver somniferum*—the symptoms and effects produced in those who indulge in this drug habitually were fully described. The method of preparation of the beverage and mode of its consumption by the addicts were also discussed. Usually 3 or 4 full-grown capsules are soaked in water for a few hours and are macerated and rubbed with fingers. The decoction thus prepared when taken is quite sufficient to produce intoxicating effects. The effects produced by the beverage thus prepared are somewhat different from those produced by opium. It was therefore necessary to find out if the alkaloids contained in the capsules were the same as opium and if so the variations in their quantities. We were unable to find any record in the literature excepting a very old note by Lyons of Bombay (1879) who determined the total amount of alkaloids in poppy capsules obtained from Malwa. More recently Mossler (*Pharm Zeit*, 1914, **59**, 600) studied the amount of morphine only in specimens of poppy capsules grown in Europe. No work appears to have been done on this subject and we undertook a number of analyses to work out the proportions of alkaloids. We realized from the beginning that the subject is very vast and unless one carries out a considerable number of estimations on samples

of opium, as well as ripe and unripe, lanced and unlanced poppy capsules grown in the same locality, it would be difficult to come to any general conclusions. The separation and estimation of the different alkaloids is also beset with difficulties as the quantity of many of them is very small. For this reason our attention was confined to the estimation of the main alkaloids only. It must also not be forgotten that the constituent alkaloids of opium are present in different proportions at different stages in the life history of the poppy plant. These variations are due to the changes in the alkaloids during metabolism, to the action of the ferments and enzymes present in the plant or to other biochemical changes. Again, the constituent alkaloids of opium differ widely in their proportion in the produce of different localities. Therefore to get an exact idea as to whether the proportion of the different alkaloids present in opium are the same as are obtainable from poppy capsules (ripe or unripe) lanced or unlanced, estimations of the total and the main constituent alkaloids of capsules of different localities and in different stages of their growth, as well as of the samples of opium collected from the same plot seems to be necessary.

There is no suitable method for the estimation of the total alkaloids of opium, much less of the capsules. It is for this reason that the criterion with which the different pharmacopœias have to be satisfied is merely the estimation of morphine—pharmacologically the most active alkaloid of opium—to lay down the standard of quality of this drug and its preparations. It has, however, been found that all the alkaloids of opium possess a certain amount of physiological activity and unless a suitable method for the determination of the total alkaloids is forthcoming the assay of opium will remain unsatisfactory. The feeling that estimation of morphine alone does not correctly indicate the medicinal value of opium has been growing in recent years and has given rise to what is called 'normal opium,' which is standardized for its four important constituents, viz., morphine 12 per cent, narcotine 6 per cent, codeine 1 per cent and meconic acid 5 per cent. In this paper we give the results of analysis of a number of samples which were examined with a view to explain the variations in the symptoms produced.

EXTRACTION OF THE ALKALOIDS

The following method of extraction and isolation of the total alkaloids from poppy capsules was adopted. This method, though it cannot be said to give absolutely correct results, is satisfactory for all practical purposes.—

The air-dried capsules (without seeds) were coarsely powdered and then extracted with hot 90 per cent alcohol in an extraction apparatus till complete exhaustion. When 10 c.c. of the last portion of the alcoholic extract gave no residue nor any appreciable precipitate with Mayer's reagent, the alcohol was distilled off. The semi-solid extract was then mixed with water, warmed for some time to remove the last trace of alcohol. The residue was then extracted with fresh portions of hot

water and finally with 1 per cent hydrochloric acid till the last portion of the acid extract gave no precipitate with Mayer's reagent. The whole of the solution was then filtered off, concentrated if too bulky, and treated with a little petroleum ether in a separator to remove fats, etc. No alkaloid was found in the ether and it was rejected. The acid solution was then divided into two portions and treated in the two following ways. In the first place it was made alkaline with NH_4OH till complete precipitation and kept overnight. The precipitate was then filtered on a counterpoised filter paper and washed thoroughly, dried and weighed. The filtrate and the washings were again made just acid with dilute HCl , shaken up with excess of chloroform, made alkaline again with dilute NaOH and vigorously shaken. This treatment with chloroform was repeated several times till the last portion of the chloroform solution left no appreciable residue nor responded to the Mayer's test. The whole chloroform solution was then washed with a little water in a separator, filtered and distilled. The weight of the precipitate together with the weight of the residue obtained from chloroform gives the amount of total alkaloid*. When the amount of morphine is high the first method is preferable to expedite operation, but when the morphine content is low, as in the case of the capsules, and the alkaloid is freshly precipitated 4 to 5 times in the presence of chloroform, no loss of morphine need be feared. It has been found that water extracts the whole of the alkaloids

TABLE I
Total alkaloids (unlanced and lanced capsules)

Weight in grammes	Alcoholic ext per cent	Total alkaloids per cent	Localities	REMARKS
200	7.96	0.54	} Lyalpur	Unlanced
1,000		0.44		
200	9.08	0.61	} Jullandhar	"
1,000		0.56		
200	9.76	0.605	} Hoshnarpur	,
1,000		0.58		
200	8.31	0.22	} Ghazipur	Lanced
1,600		0.187		
200 (average size)		0.150		

* In the second method, without precipitating the alkaloid and weighing the precipitate separately, the whole of the solution was made alkaline with NH_4OH and taken up with chloroform.

from the alcoholic extract and no use of acid is necessary. Similarly, the whole of the alkaloids in the capsules can be extracted by means of maceration and squeezing with 4 to 5 changes of water and thus the 'Sherbat' or decoction prepared by the addicts contains nearly the whole of the alkaloids present in the capsules.

In all the above estimations the selected dry capsules (each weighing not less than 3 grammes) were taken. Samples weighing less than 2 grammes gave not more than 0.2 per cent of total alkaloids.

From a perusal of the table it will be seen that on an average there is about 0.4 to 0.6 per cent of total alkaloids in the unlanced capsules and from 0.15 to 0.22 in selected lanced capsules. If the Ghazipur lanced capsules are taken as a typical example it would appear that about one-third of the total alkaloids remains in the capsules after removing the inspissated juice by lancing. Dry opium, when treated as above, gives on an average 30 per cent of crude alkaloids (see also 'The Total Alkaloids of Opium' by J. N. Rakshit) and if we calculate 1 gramme of crude alkaloid equal to 3 grammes of opium, it can be stated that unlanced capsules contain about 2 grammes (30 grains) and lanced capsules about 0.6 gramme (9 grains) of dry opium per 100 grammes (or 25 to 30 capsules without seeds). Computing in this way it may be said that 3 or 4 capsules may contain as much as 6 grains of ordinary opium which would be quite sufficient to produce intoxicating effects. It is possible that the large amount of water with which the capsules are taken helps in quick absorption and stronger effects. It is premature to assert definitely any relationship between the proportion of the alkaloids in the lanced and unlanced capsules without examining both kinds of the same locality, but there is no doubt that a fair quantity of alkaloids remain in the capsules after lancing. As regards the main alkaloids (viz., morphine, narcotine and codeine) present in the capsules, various methods of separation and estimation, both from the crude alkaloids obtained from the capsules as well as from the extract, were tried. The following is an outline of the method which gave satisfactory results—

As already observed, water extracts nearly the whole of the alkaloid from an alcoholic extract, 2 kilos of the dried capsules were first exhausted with hot 90 per cent alcohol. The alcohol was distilled off and the residue was extracted with hot water several times, filtered and the whole solution made up to 1,000 c.c. Aliquot parts of 250 c.c., 100 c.c., 100 c.c. of the solution representing 500, 200, 200 grammes of capsules were evaporated to dryness and the quantity of morphine, narcotine and codeine was respectively determined in the residues. For the estimation of morphine in all the samples excepting the Ghazipur (lanced ones), the B. P. method modified by A. B. Stevens was followed. This method was not very suitable for the lanced capsules as the amount of morphine is very small, Eaton's Assay Method for Camphorated Tincture Opium (*Bureau of Chemistry Bull.*,

1911, 37, 188) was therefore adopted. The estimation of narcotine—the residue obtained by evaporation of the second aliquot portion (100 c c) was dissolved in dilute hydrochloric acid, filtered, any large excess of acid with sodium carbonate neutralized and to it was then added a concentrated solution of sodium acetate. It was then filtered after keeping it overnight and washed lightly. The precipitate on the filter paper was treated with dilute hydrochloric acid and warmed on the water-bath till it dissolved, the solution filtered, the filter paper washed. The whole of the acid solution was then transferred to a separator, made slightly alkaline with ammonia and again faintly acid with acetic acid and then well shaken with benzene several times. The benzene solution was then washed with a small quantity of water, then distilled and finally evaporated to dryness. The residue, the whole of which crystallized at once from alcohol, weighed, gives the amount of narcotine.

The codeine was estimated according to the method suggested by Mr Rakshit (*Analyst*, December 1921) from another 100 c c of the aliquot portion.

TABLE II.

Locality	Per cent morphine in capsules	Per cent morphine in total alkaloid	Per cent narcotine in capsules	Per cent narcotine in total alkaloid	Codeine in capsules	Per cent codeine in total alkaloid
Lyalpur	0.042	6.6	0.205	34	0.11	18.3
"	0.048	8.0	0.182	30	0.102	17.1
Jullandhar	0.031	4.6	0.146	24	0.084	14
"	0.025	3.5	0.128	23	0.071	12.8
Hoshiarpur	0.126	21.1	0.175	35	0.078	15.2
"	0.141	24.3	0.168	33	0.082	16
Ghazipur (lanced)	0.015	6.8	0.061	27	0.032	14.5
"	0.011	5.4	0.063	29	0.030	13.6
Opium (average composition)		30		30—45		12—15

If we take the proportion of total alkaloids in crude opium to be 1·3 the calculated result in the capsules compared with crude opium would be as follows —

TABLE III

	Per cent of morphine	Per cent of narcotine	Per cent of codeine
Lyalpur	2·2	11·3	6·1
„	2·6	10	5·7
Jullandhar	1·5	8	4·6
„	1·2	7·6	4·2
Hoshiarpur	7	11·6	5·06
„	8·1	11	5·3
Ghazipur (lanced)	2·2	9	4·8
Opium (average composition)	10	10—15	4—5

Comparing the percentages of morphine, narcotine and codeine present in the total alkaloids of the poppy capsules with those of the same alkaloids as are generally present in the alkaloids of opium, we find that the amount of morphine is far below, while that of narcotine and codeine are never lower, if not higher than the average amount of these alkaloids present in opium. The only exception is the Hoshiarpur samples in which the above-mentioned alkaloids do not show much variations from those of the average quantities present in opium. Most probably this is due to the fact that the capsules must have been plucked when fully developed and ready for the lancing operation (i.e., were not allowed to ripen) and so further changes in the alkaloidal content have been prevented. The fact that there is a deficiency of morphine in the fully ripe poppy capsules was also observed by Mossler, who stated 'that there is a considerable diminution of morphine from 0·1369 per cent in air-dried capsules falling to 0·053 per cent in ripe capsules' (*Pharm Zeit*, 1914, **59**, 600). Another fact observed is that the percentage of the total alkaloids in the capsules, though of different localities, lies within narrow limits.

DISCUSSION

It is well known that activity of opium depends on the alkaloids it contains, of which as many as twenty have been isolated. With regard to the chemical composition and pharmacological action they all fall under two groups

(1) Those containing phenanthrene nucleus represented by morphine, codeine, thebaine, and five artificial alkaloids heroin, diionine, peionine, apomorphine, and apocodeine (2) Those containing the iso-quinoline nucleus which is present in narcotine, papaverine and narcine The average sample of total alkaloids contains in 100 parts (a) morphine 62 parts, codeine 3 parts, thebaine 3.25 parts and (b) narcotine 22 parts, papaverine 3.5 parts, narcine 1.9 parts, the balance are the other alkaloids

The chief action of these alkaloids is on the nervous system, their effects on other structures being mainly indirect Generally the natural phenanthrene alkaloids act chiefly on the nervous system and the iso-quinoline alkaloids more strongly on the autonomic system The action of opium is so denominated by that of morphine as to coincide with it in the main The iso-quinoline alkaloids merely modify the action of the total drug by their relaxing effect on the smooth muscles and their sedative effect on the autonomic system Opium is thus more constipating than morphine and is more effective on painful spasmodic affections of the smooth muscles

The alkaloids of opium are more or less narcotic and convulsant in their action but the latter group occur in smaller quantities and their action is dominated by the former group The exact differences between the action of morphine, opium and other mixtures of alkaloids introduced in therapeutics (e.g., pantopan, narcophine, etc.) have not been worked out It is known that narcotine, which is not a very active alkaloid, increases the toxicity of morphine and codeine The differences in the symptoms and effects produced by opium and poppy capsules can all be explained by large quantities of narcotine and codeine which are contained in them

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CHANGES IN THE PHYSICAL PROPERTIES OF KALA-AZAR SERUM WITH TREATMENT AND ITS RELATION TO THE FORMOLGEL REACTION

BY

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[Received for publication, April 20, 1931]

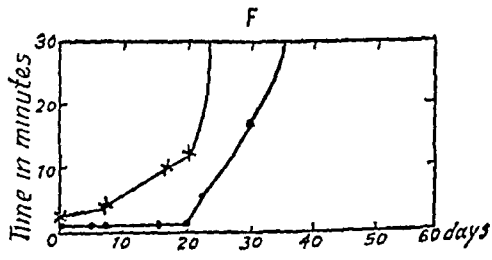
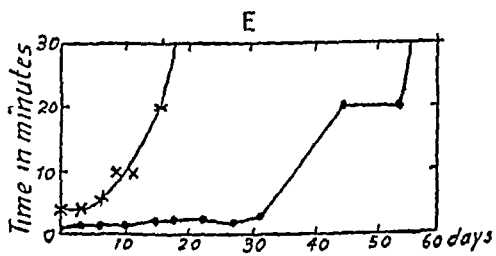
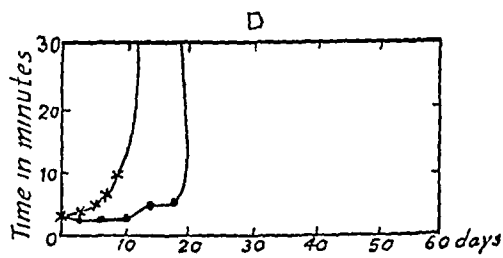
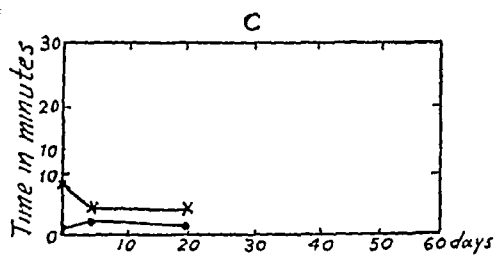
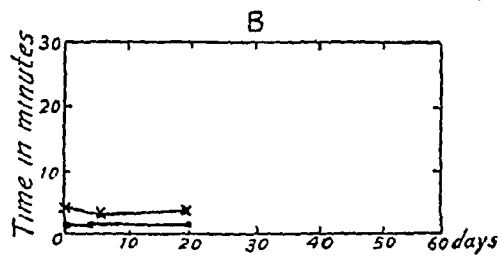
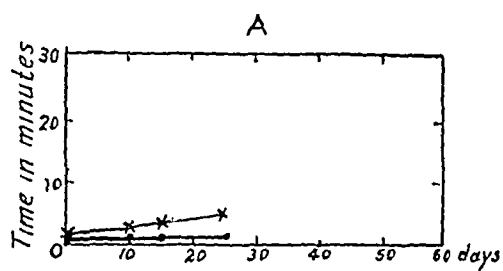
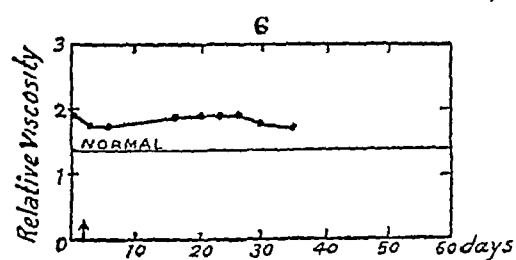
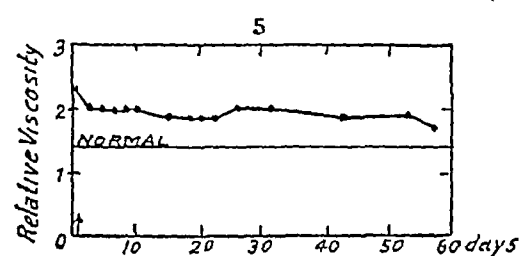
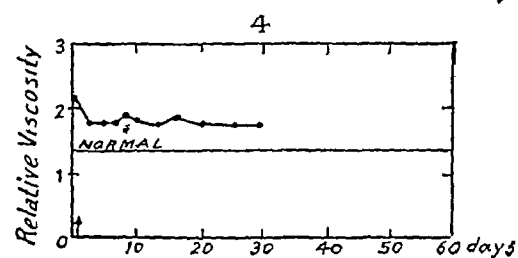
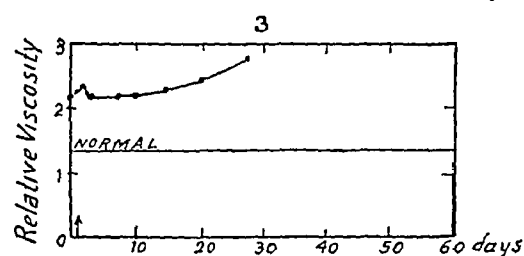
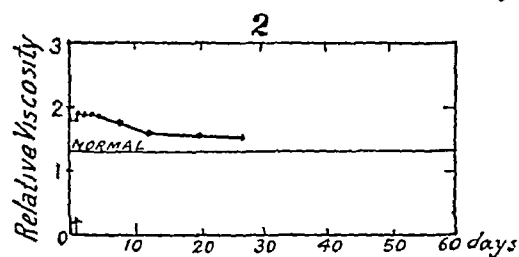
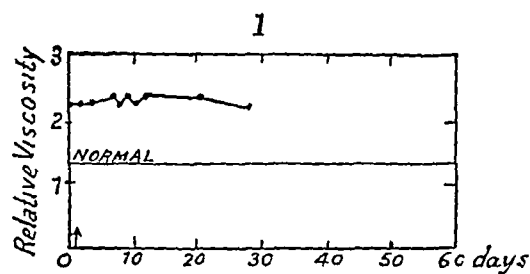
INTRODUCTION

THE present work was undertaken originally with a view to note the changes of viscosity of the serum from the blood of kala-azar patients with the course of injections of antimony preparations, and to see when this property approaches its normal value during the treatment. As the measurement of viscosity of serum is very simple, we thought that it would be advantageous to use this property as diagnostic of the end point in the cure of kala-azar during the course of the treatment. Such serological cure point has already been established by Lloyd and Paul (1928) on the basis of the albumin globulin ratio and the euglobulin content and our idea was simply to supplement their results with a simpler and quicker procedure. But unfortunately almost all of the patients left the hospital before the serum assumed its normal aspect and thus, though the examinations were carried at regular intervals for about two months, in most of our cases from the beginning of the treatment, the value for viscosity of the serum did not approach normality.

Simultaneously, with these measurements, we made a quantitative study of the formolgel reaction. In view of the relation existing between viscosity and the

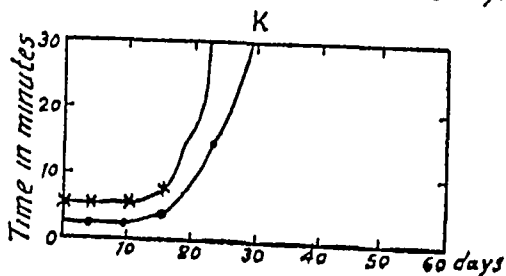
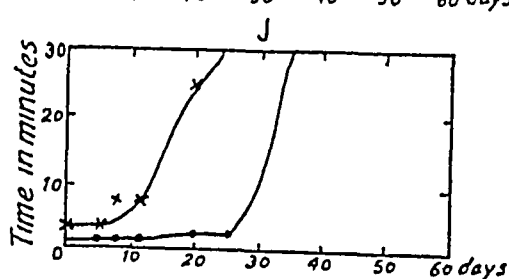
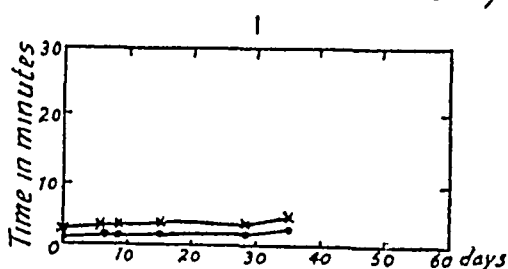
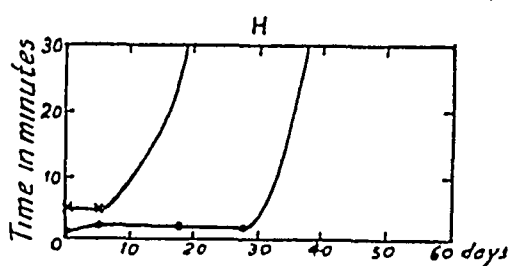
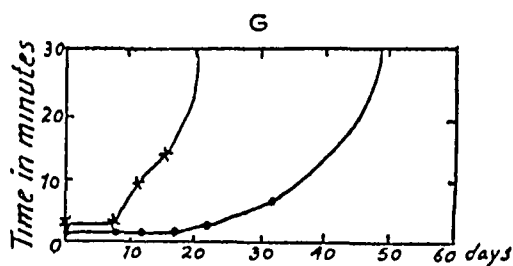
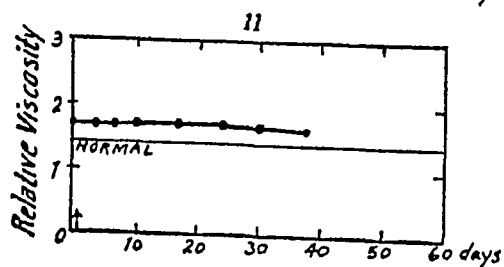
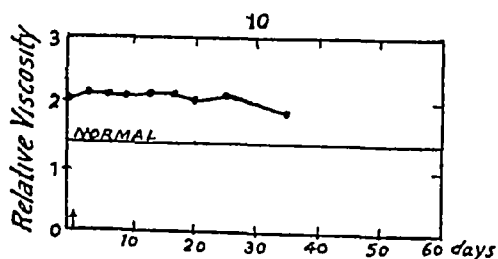
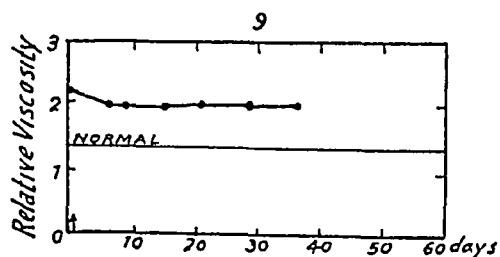
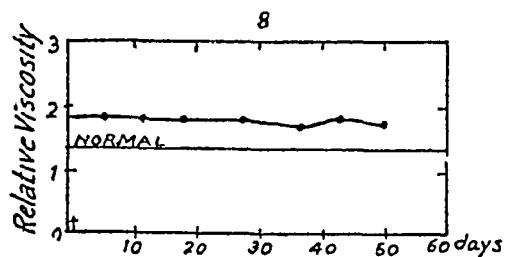
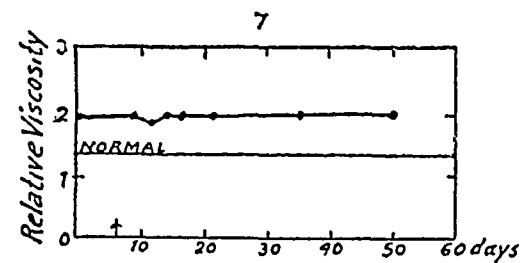
GRAPHS 1—6

GRAPHS A—F



GRAPHS 7—11

GRAPHS G—K



REFERENCES

- Arrow from the X-axis denotes the first date of injection
 × denotes time of complete opacity
 • denotes time of gelation
- } in the Graphs A to K

times of gelation and of complete opacity, we thought these results to be worth communicating. Incidentally, the cause of the formation of these proteins has also been discussed in the light of blood counts during the course of the treatment in some of the cases.

EXPERIMENTAL

The technique for measurements of viscosity and times of gelation and complete opacity has been described in our previous papers (1929*a* & *b*). The results are all graphically represented. Graphs 1 to 11 show the change of viscosity with the course of treatment with the pentavalent antimony preparations. Graphs A to K represent the times of gelation and complete opacity with the treatment and correspond to viscosity Graphs 1 to 11 respectively. An arrow from the *X*-axis shows the date of the first injection. The patients chosen were all from Dr. L. E. Napier's kala-azar ward in the Carmichael Hospital for Tropical Diseases, Calcutta, and in each case leishmania had been demonstrated in the peripheral blood by spleen puncture. The method of treatment adopted was pentavalent antimonial preparations by intravenous injections in increasing doses, in the first three cases the method consisted of two courses of four injections on consecutive days and the interval between them was about a week, for the rest a concentrated course for six consecutive days was followed.

DISCUSSION

The curves for viscosity (except Graph 3) show that the viscosity of sera do not in some cases change appreciably with the course of the treatment for about two months and in others practically remains constant and in both cases is always above the normal. Graph 3 shows a decided rise in its value, but this was an abnormal case and its corresponding formolgel reaction (Graph C) shows that time of complete opacity also diminished. Clinically this was actually a case of relapse and the abnormality in viscosity and formolgel reaction is thus explained. The other three cases (Graphs 1, 2, 9 and A, B, G), though not abnormal to such an extent, show a poor response to treatment so far as it can be gathered from the viscosity values and the times of gelation and complete opacity. It should be borne in mind that in the first three cases (1, 2, 3, A, B, C) the method of treatment was different from the rest and this method has already been found to be inferior to a concentrated course of injections for six consecutive days by Lloyd, Napier and Paul (1929), i.e., the onset of cure comes off at a later stage. Besides this, clinically the progress of the patient (Graphs 2 and B) was not satisfactory, while that of the other one (Graphs 3 and C) was not particularly good. The fourth patient (Graphs 9 and I) had a concentrated course of six injections of amino-stiburea and his clinical progress was apparently satisfactory, but he had also anæmia and therefore his case cannot be strictly compared with other

cases of kala-azar For the rest the clinical progress was good and is in agreement with the results obtained

The reason for the value of the viscosity remaining constant is not far to seek in the light of the results on the protein contents already published by Lloyd and Paul (*loc cit*) It is well known that viscosity mainly depends on the concentrations of the proteins in the serum, while the other constituents have only a slight effect These authors have pointed out that, on the first stage, the pseudoglobulin shows an enormous and very rapid fall, while the albumin shows an equally sudden rise, so that the lowering of the viscosity due to the former is compensated by its increase due to the rise in the latter constituent At the second stage, however, the albumin content remains constant, whereas the pseudoglobulin increases and the euglobulin decreases, so that viscosity ought not to diminish much Mass for mass, the viscosity of euglobulin is higher than that for pseudoglobulin, but the rise in the concentration of the former might be greater than the fall in the concentration of the latter, where this does not take place the viscosity should diminish and at a later stage we find a tendency for this property to diminish, though we have not been able to proceed with any case more than two months

Comparing these curves with the times of gelation and complete opacity it is found that in general there is no close agreement although the gelation time remains constant for about twenty to thirty days from the beginning of the treatment The time of complete opacity, however, begins to increase much earlier than the time of gelation in each and every case This constant slower appearance of opacity after gelation suggests that the formation of opacity is not the primary reaction when formalin is added to the kala-azar serum, but obviously a process secondary to gel-formation The authors in a previous paper (1929a) have shown that for a particular kala-azar serum, there is a definite pH at which the time of gelation and complete opacity are minimum In some cases they occur at the pH value 6.9 and in others at the pH value 7.27 Also the rate of decrease of opacity is much greater than the rate of decrease of gelation on the alkaline side, whereas the rate of decrease of gelation is greater than the rate of decrease of opacity on the acid side The difference in the rate of variations of these two times points strongly to the suggestion that gel-formation and development of opacity are caused by two different proteins, but the coincidence of the pH value of the minimum times of gel-formation and opacity suggests that both the processes are due to one and the same protein But this same minimum pH value of both the processes was explained by assuming that during the process of gel-formation, a large quantity of water is absorbed by the gel and therefore this process of gelation helps the secondary process of precipitation of the other proteins, specially euglobulin The above fact, that opacity comes always after and is secondary to gelation with the treatment, supports the views just mentioned.

times of gelation and of complete opacity, we thought these results to be worth communicating. Incidentally, the cause of the formation of these proteins has also been discussed in the light of blood counts during the course of the treatment in some of the cases.

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DISCUSSION

The curves for viscosity (except Graph 3) show that the viscosity of sera do not in some cases change appreciably with the course of the treatment for about two months and in others practically remains constant and in both cases is always above the normal. Graph 3 shows a decided rise in its value, but this was an abnormal case and its corresponding formogel reaction (Graph C) shows that time of complete opacity also diminished. Clinically this was actually a case of relapse and the abnormality in viscosity and formogel reaction is thus explained. The other three cases (Graphs 1, 2, 9 and A, B, G), though not abnormal to such an extent, show a poor response to treatment so far as it can be gathered from the viscosity values and the times of gelation and complete opacity. It should be borne in mind that in the first three cases (1, 2, 3, A, B, C) the method of treatment was different from the rest and this method has already been found to be inferior to a concentrated course of injections for six consecutive days by Lloyd, Napier and Paul (1929), i.e., the onset of cure comes off at a later stage. Besides this, clinically the progress of the patient (Graphs 2 and B) was not satisfactory, while that of the other one (Graphs 3 and C) was not particularly good. The fourth patient (Graphs 9 and I) had a concentrated course of six injections of amino-stiburea and his clinical progress was apparently satisfactory, but he had also anaemia and therefore his case cannot be strictly compared with other

cases of kala-azar. For the rest the clinical progress was good and is in agreement with the results obtained.

The reason for the value of the viscosity remaining constant is not far to seek in the light of the results on the protein contents already published by Lloyd and Paul (*loc cit*). It is well known that viscosity mainly depends on the concentrations of the proteins in the serum, while the other constituents have only a slight effect. These authors have pointed out that, on the first stage, the pseudoglobulin shows an enormous and very rapid fall, while the albumin shows an equally sudden rise, so that the lowering of the viscosity due to the former is compensated by its increase due to the rise in the latter constituent. At the second stage, however, the albumin content remains constant, whereas the pseudoglobulin increases and the euglobulin decreases, so that viscosity ought not to diminish much. Mass for mass, the viscosity of euglobulin is higher than that for pseudoglobulin, but the rise in the concentration of the former might be greater than the fall in the concentration of the latter, where this does not take place the viscosity should diminish and at a later stage we find a tendency for this property to diminish, though we have not been able to proceed with any case more than two months.

Comparing these curves with the times of gelation and complete opacity it is found that in general there is no close agreement although the gelation time remains constant for about twenty to thirty days from the beginning of the treatment. The time of complete opacity, however, begins to increase much earlier than the time of gelation in each and every case. This constant slower appearance of opacity after gelation suggests that the formation of opacity is not the primary reaction when formalin is added to the kala-azar serum, but obviously a process secondary to gel-formation. The authors in a previous paper (1929a) have shown that for a particular kala-azar serum, there is a definite pH at which the time of gelation and complete opacity are minimum. In some cases they occur at the pH value 6.9 and in others at the pH value 7.27. Also the rate of decrease of opacity is much greater than the rate of decrease of gelation on the alkaline side, whereas the rate of decrease of gelation is greater than the rate of decrease of opacity on the acid side. The difference in the rate of variations of these two times points strongly to the suggestion that gel-formation and development of opacity are caused by two different proteins, but the coincidence of the pH value of the minimum times of gel-formation and opacity suggests that both the processes are due to one and the same protein. But this same minimum pH value of both the processes was explained by assuming that during the process of gel-formation, a large quantity of water is absorbed by the gel and therefore this process of gelation helps the secondary process of precipitation of the other proteins, specially euglobulin. The above fact, that opacity comes always after and is secondary to gelation with the treatment, supports the views just mentioned.

times of gelation and of complete opacity, we thought these results to be worth communicating. Incidentally, the cause of the formation of these proteins has also been discussed in the light of blood counts during the course of the treatment in some of the cases.

EXPERIMENTAL

The technique for measurements of viscosity and times of gelation and complete opacity has been described in our previous papers (1929*a* & *b*). The results are all graphically represented. Graphs 1 to 11 show the change of viscosity with the course of treatment with the pentavalent antimony preparations. Graphs A to K represent the times of gelation and complete opacity with the treatment and correspond to viscosity Graphs 1 to 11 respectively. An arrow from the *X*-axis shows the date of the first injection. The patients chosen were all from Dr. L. E. Napier's kala-azar ward in the Carmichael Hospital for Tropical Diseases, Calcutta, and in each case leishmania had been demonstrated in the peripheral blood by spleen puncture. The method of treatment adopted was pentavalent antimonial preparations by intravenous injections in increasing doses, in the first three cases the method consisted of two courses of four injections on consecutive days and the interval between them was about a week, for the rest a concentrated course for six consecutive days was followed.

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The next question arises whether the protein, which is responsible for gel-formation, is the same as or other than euglobulin. The minimum time of gelation near about the pH value 7 suggests that this protein is different from euglobulin. The iso-electric point of euglobulin is according to all standard authors 5.5. The gel-protein is obviously very much similar to euglobulin in its solubility and other physical and chemical properties, so that when we precipitate the proteins from the serum by 33 per cent ammonium sulphate or some such reagent, both the proteins are precipitated. According to Lloyd and Paul on about the 23rd day of the treatment the euglobulin begins to diminish and it is worthy of note that from about this time onward the gelation time is also increased. It cannot be due to the diminution in euglobulin, for then the minimum time of gel-formation ought to have been at 5.5, which is undoubtedly the iso-electric point for this protein. Hence we are inclined to the view that the protein which is responsible for gel is different from euglobulin.

It is also well known that in other pathologic sera, such as those from malarial and syphilitic patients, there is change from a more or less semi-clear to a clear gel with formalin according to the type of the diseased sera used. This fact points to the existence of this gel protein in other sera too and it is very likely that it is present in very small amounts in normal sera. The formation of a clear or semi-clear gel in other diseased sera points also to the source from which these proteins are derived. Compared to malarial and syphilitic patients, kala-azar patients have got decidedly more leukopœnia. Hence the destruction of the white blood corpuscles go hand in hand with the existence of a protein which is not responsible for gel but for opacity. On the other hand, in all of these diseases, there is a low erythrocyte count and this suggests that the presence of the protein, which is responsible for gel, is accompanied by the destruction of the red blood corpuscles. Accordingly, we thought it proper to study the R B C and W B C counts of kala-azar patients with the times of gelation and complete opacity in a few cases. The results are given in the following tables —

TABLE I

Days	Time of gelation.	Time of complete opacity	Total R B C.	Total W B C
0	$\frac{1}{2}$ minute	2 minutes	2,920,000	2,800
6	" "	" "	2,980,000	2,900
13	" "	" "	2,380,000	2,960
18	" "	$1\frac{1}{2}$ "	2,230,000	2,500

TABLE II

Days	Time of gelation	Time of complete opacity	Total R B C	Total W B C
0	$\frac{3}{4}$ minute	7 minutes	3,800,000	3,120
6	" "	10 "	1,050,000	1,368
13	" "	15 "	4,100,000	5,000

TABLE III

0	$\frac{1}{2}$ minute	1 $\frac{1}{2}$ minutes	3,250,000	2,800
6	" "	" "	3,060,000	3,120
13	" "	" "	" "	"
27	" "	3 $\frac{1}{2}$ "	3,170,000	3,500

A perusal of these tables will show that there is somewhat of a parallelism between the time of gelation and the R B C count and the time of complete opacity and the W B C count. Provisionally, therefore, the suggestion, that the gel-protein is other than euglobulin and is concomitant partly at least with the destruction of the R B C, is in agreement with experimental facts. Finally, as to the nature of the protein, it appears that it is similar to fibrin globulin as described by Hammersten (1914), for the quantity of fibrin obtained on coagulation of blood is always smaller than the amount of fibrinogen from which the fibrin is derived and there is always a small amount of protein substance in the solution. It is, therefore, not improbable that the fibrin coagulation, in accordance with the views first proposed by Dennis, is a cleavage process in which the soluble fibrinogen is split up into an insoluble protein—the fibrin—which forms the chief mass, and a soluble protein substance which is produced in small amounts and is a globulin-like substance. This globulin-like substance is expected to be in large quantity in the kala-azar sera, as the coagulation time is increased in these cases. Moreover, the iso-electric point of fibrin is also 6.8, the same pH at which the iso-electric point of the gel-forming protein is located. But though these suggestions are the most probable that we can think of, it is to be noted the last one on the nature of the protein is more or less of a hypothetical character. Our best thanks are due to Dr L. E. Napier, M.R.C.S., L.R.C.P., for giving us facilities for obtaining blood from patients under his treatment and information about their clinical progress.

CONCLUSIONS

1 The viscosity of sera from the blood of kala-azar patients does not change appreciably with the course of treatment by pentavalent antimony compounds for about two months

2 The times of gelation and complete opacity of the kala-azar sera with formalin during the course of treatment vary widely The time of complete opacity begins to increase from about 10 to 15 days after treatment, whereas the time of gelation increases about 20 to 30 days after treatment

3 The suggestion that the gel-forming protein is other than euglobulin is in agreement with facts, and has been fully discussed from all points of view

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BLOOD FINDINGS IN NORMAL MONKEYS

BY

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[Received for publication, April 24, 1931]

WHILST trying to produce anæmia in monkeys by dietetic experiments normal blood findings were sought for, but finding the literature on that subject scant the figures in the table were worked out from a series of twenty-seven adult monkeys of both sexes of the species *Macacus sinicus* and *Macacus rhesus*

This paper is published in the hope that it may be of some use to research workers in India experimenting on monkeys

The counts for both red and white cells were done on standard Levy-Hausser counting chambers, and standard Trenner automatic pipettes were used for dilution. The diluent used for the red cells was Hayem's solution, further diluted with distilled water in the proportion of 3 : 2. For the white cells 2 per cent oxalic acid was used.

The hæmoglobin concentration was compared on a Leitz colorimeter against a standard of Acid Hæmatin prepared from dog's blood, which was estimated by the oxygen combining capacity method of Van Slyke.

The mean diameter of red cells was determined on a modified Young's criometer (Preston) as described by Pryce (1929).

The blood picture did not differ from that of the normal human being, except that the red cells were slightly smaller in size (mean diameter 6.4μ).

The differential count showed a preponderance of lymphocytes over the myeloid cells. This statement is borne out by Fox (1923).

In conclusion I wish to thank the Superintendent of the Victoria Gardens, who so kindly placed at my disposal the necessary material to work on.

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TABLE
Blood findings in 27 normal monkeys

RED BLOOD CELLS		HÆMOGLOBIN		WHITE BLOOD CELLS	
IN MILLIONS PER C MM	DIAMETER IN μ	IN GRAMMES PER CENT	DIFFERENTIAL COUNT PER CENT	TOTAL COUNT	
6.43	6.4	12.53	35	Minimum value	2
				Maximum value	2
				Standard deviation of mean	61
				Mean value	35
7.88	6.9	13.80	61	Minimum value	2
				Maximum value	2
				Standard deviation of mean	61
				Mean value	35
5.17	6.1	11.17	2	Minimum value	2
				Maximum value	2
				Standard deviation of mean	61
				Mean value	35

ON THE MORPHOLOGY OF THE TERMINAL SEGMENTS OF PSYCHODIDÆ LARVÆ AND THEIR TAXONOMIC IMPORTANCE

(WITH A SHORT COMPARATIVE ACCOUNT OF THE MICROSCOPIC
STRUCTURE OF THE PSEUDO-LEG OF *PHLEBOTOMUS*
ARGENTIPES ANN AND BRUN)

BY

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[Received for publication, July 20, 1931]

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- 3 TECHNIQUE
- 4 GENERAL STRUCTURE AND FUNCTION OF THE TERMINAL SEGMENTS OF PSYCHODIDÆ LARVÆ
AND THEIR MODIFICATIONS
 - (a) Terminal segment of the mature larva of *Telmatoscopus albipunctatus* Willst =
(*meridionalis* Eat) Brun
 - (b) Terminal segment of the mature larva of *Psychoda alternata* Say = (*sexpunctata* Curt) Brun
 - (c) The two terminal segments of the mature larva of *Phlebotomus argentipes* Ann and Brun
 - (d) The two terminal segments of the mature larva of *Phlebotomus papatasi* Scop
 - (e) The two terminal segments of the mature larva of *Phlebotomus minutus* Rond (*sensu lato*)
- 5 A SHORT COMPARATIVE ACCOUNT OF THE MICROSCOPIC STRUCTURE OF THE PSEUDO LEG OF
Phlebotomus argentipes
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1 INTRODUCTION

IN view of the rôle played by the members of the family Psychodidæ, more especially of the genus *Phlebotomus*, as vectors of various diseases the systematic study of the immature stages of these flies is of importance. Unfortunately, literature on this subject is surprisingly scanty thus Perfiljew (1928) very aptly remarked ' Ueber *Phlebotomus*-larven liegen in der Literatur sehr spärliche Angaben vor ' and I am in full agreement with him

In a previous communication to *Nature* (1930) a short summary of the present work was indicated. Although, at first, I intended to deal with the terminal segments of the larvæ of most of the common Indian Psychodidæ, unfortunately with the limited resources at my disposal, I failed to secure sufficient numbers of gravid imagines of the other species for this type of work. I propose, therefore, to deal at present with the structural modifications of the caudal segments of three species belonging to the genus *Phlebotomus* and of two belonging to the genera *Psychoda* and *Telmatoscopus* *.

These species occur in sufficient numbers in and around Calcutta to make it possible to carry out a series of observations and to arrive at a conclusive result. The author, while engaged in the breeding of sandflies for experimental purposes, had an excellent opportunity of studying the immature stages of the local forms.

I should like also to record herein my observations on the microscopic structure of the 'pseudo-leg' † of the larvæ of *Phlebotomus argentipes* Ann and Brun which is characteristic of the larvæ of the genus.

I desire to take this opportunity of expressing my thanks to Lieut-Colonel H. W. Acton, Director, Calcutta School of Tropical Medicine and Hygiene, for kindly lending me his able artist Mr. H. M. Roy who finished my pencil sketches with his usual accuracy and skill, to the Librarian and his assistants of the School for finding for me some rare foreign literature, and last but no less sincerely to the insect collectors of the Inquiry for the painstaking nature of their work. I should like also to record herein my indebtedness to my chief Dr. L. E. Napier, officer in charge, Kala-azar Inquiry, for encouraging me by reporting on the progress of the work in his various official reports. Specimens of the sub-family Psychodinae were identified by Dr. A. L. Tonnon, Senior Ecologist and Curator, Council of Scientific and Industrial Research, Australia, for which I am deeply indebted to him.

2 CRITICAL REVIEW OF PREVIOUS WORKS

The imagines of the family Psychodidæ (except the sub-family Psychodinae) ‡ have long been recognized as blood-suckers and as such attracted the attention of eminent morphologists and systematists such as Grassi, Newstead, Christophers, Shortt and Barraud, Annandale and Brunetti, Perfiljew, Popov, Peterson, Shannon, Delponte, Parrot and França, Patton and Hindle, Nitzulescu, Dyer, Larrousse, Sinton, Adler and Theodor and a few others. Unfortunately, our systematic knowledge of the immature stages of the group has not progressed in parallel lines. This is perhaps due in the first place to the secluded nature of the breeding

* Further observations of this nature will in future be made as time and opportunity occurs, and as specimens in sufficient numbers are forthcoming.

† I suggest the non committal name of 'Pseudo legs' for these temporary structures which are only of importance to the active larva and disappear in the pupa.

‡ One species of *Pericoma*, viz., *P. townsillensis*, has been recorded as a blood sucker.

places of some members of the family, especially, of the genus *Phlebotomus* and secondly to the fact that unless and until the complete life-history of each species is studied thoroughly in the laboratory, one is not in a position to assign an egg, a larva or a pupa to a particular species. Works on the immature stages of particular species have unfortunately only appeared sporadically in scattered journals. These are important only from the morphological point of view and as will be seen presently no definite attempt has as yet been made to correlate the systematic positions of the immature stages of the members of the family.

Grassi (1907) in his monograph deals extensively with the bionomics and certain aspects of morphology of the immature stages of *Phlebotomus papatasi* Scop. Unfortunately, he is not sure about the age of the larvæ dealt with, and describes them as 'relativamente piccola' and 'giunta al massimo sviluppo' or some such vague terms.¹ His figures of the terminal segments although characteristic for the genus, cannot be taken as accurate since the structure of the different sclerites, the characteristic pigmentation and the origin of the typical spines of the sclerites have not been shown with sufficient clarity.

Newstead (1911-12) gives a more or less detailed description of the caudal bristles of the last instar larva of *Phlebotomus papatasi*. Howlett (1915-16) says that the eggs or young larvæ afford easy means of identification. His figures of the young larvæ are, however, not very encouraging. Again he bases his classification of the larvæ on the relative length of the tail bristles as compared with the head and the body together with the structure and shape of the dorsal body bristles generally of the posterior parts of the abdomen of young and adult larvæ. These differentiating characters may, to some extent, prove useful for laboratory-bred specimens, but whether they would be of any practical value in identifying larvæ secured by the flotation method of Young, Richmond and Brendish is still open to question since the spines which are generally plumose may lose their contour and shape by the unavoidable rough procedure, the tail bristles are also liable to breakage, and thus give a wrong impression of their relative size. Howlett further adds in the same paper that the larva cannot burrow, this observation of his should be taken with some reserve since Shortt, Smith and Swaminath (1930) definitely remark that in their search for their food the larvæ burrow into loose soil. The same author again records the curious habit of shamming death on the part of the larva on the approach of danger. During my continued experience for 2-3 years in breeding sandflies daily, I do not remember a single occasion on which I noticed such phenomenon although it is usual for me to turn over the soil in the breeding pots. As noticed by Howlett it is not unusual for the older and mature larva owing to the great accumulation of fat to remain quiescent for hours in the prepupal stage. Howlett in the same paper remarks again that the influence of temperature has a

* Grassi himself complains of shortage of material

definite bearing on the differentiation of the species without, however, adducing any experimental evidence in support and seems to think that in this respect *Phlebotomus argentipes* is more or less intermediate between *P. papatassu* and *P. minutus** With regard to this remark of Howlett, I should like only to add here that I have been successful in breeding the three species in question at a room temperature ranging between 28°–31°C Whittingham and Rook (1922-23) describe rather fully the different stages of the larvæ of *Phlebotomus papatassu*. Their figure of the mature larvæ, however, does not show clearly the characteristic shape or pigmentation of the sclerites of the caudal segment with the typical hairs and spines on it Seguy (1925) gives a short description of the *Phlebotomus* larva and a half schematic structure of head of the ventral and lateral sides Perfiljev (1928) deals with the internal anatomy and certain aspects of the external morphology of *Phlebotomus* larva His paper is thus important from the morphological point of view only Colas-Belcour (1928) in a thesis† shows the morphological differences between newly-hatched larvæ of *P. papatassu*, Scop, *P. perniciosus*, Newst, and *P. parroti*, Adl and Theo The same author further concludes that the differences between the older larvæ and pupæ of *P. papatassu* and *P. perniciosus* are less marked though those between these two species and *P. parroti*, which belongs to the *minutus* group, persist up to the last stage He thus corroborates the demarcation already established between these two groups My observations, as will be seen later on, conducted on a similar line, on *P. argentipes*, *P. papatassu* and *P. minutus* (*sensu lato*), leads practically to the same conclusion, although I am inclined to think that the differences between the older larvæ of *argentipes* and *papatassu* cannot be considered so small as Colas-Belcour opines Shevchenko,‡ in a recent paper (1930) in Russian of which only a summary in French and copies of a few figures were obtained with great difficulty, deals with the eggs and immature stages of *P. papatassu*, *P. chinensis* and *P. sergenti* The author differentiates the primary stages by means of the shape, number and structure of the spines on head, body, terminal segments and, more especially, by the length and character of the median pair of hairs of his so-called XII segment He further concludes that the specific difference is more marked in the primary stages, while with the growth of the larvæ these differentiating characters gradually vanish From my personal observations, I am led to concur with Shevchenko when he says that one is liable to err if in specific differentiation the number of spines on the terminal segment is taken into account as the sole factor As regards Shevchenko's figure of mature

* Presumably, Howlett's *P. minutus* is applicable only in a loose sense

† I have had no access to his original paper, which so far as I could gather, did not appear in any recognized entomological journal The thesis was reviewed in *Rev Appl Ent B* 10, Oct 1928 Unfortunately, the reviewer does not point out the morphological characteristics on which the classification is based

‡ The author was apparently unaware of my note in *Nature* (1930) which appeared earlier than his. His bibliography does not include my name,

larva of *P. papatassu* (Fig 17), I should like to point out that the penultimate segment shown is distinctly larger than the caudal one which is not the case at least with the Indian forms of the species. In the same figure the caudal segment is shown broader than long while the dentate marginal border of the segment is shown as straight. Again, uniform pigmentation of the two terminal segments is shown in the same figure. My observations, however, with the Indian forms of *P. papatassu* as will be seen later on are not in conformity with all these observations of Shevchenko. Besides, Shevchenko's figure lacks in the accurate delineation of the different sclerites and the points of origin of the different types of hairs and spines of the caudal segment.

3 TECHNIQUE

Specimens were provisionally identified from external characters before being put in tubes for breeding. In breeding, the technique adopted by Christophers, Shortt and Bariaud (1926) was followed. From each batch of the provisionally diagnosed species, two lots of each type, both premature and mature larval forms, were carefully collected. One of these lots was treated with 'Lacto-chloral' * and the pigmented parts were observed in reflected light. The second lot, on the other hand, was cleared in the usual way in KOH, stained with carbol-eosine and mounted in thin Euparal. The latter method was adopted to get a correct idea of the structure of the sclerites, the points of origin of the different hairs and spines and also to some extent of the depth of pigmentation. The hatched-out imagines from each batch were collected separately and their systematic positions were diagnosed and thus the previous identification confirmed. Except for the diagnosis of the females of the *minutus* group † used in the experiment, the latest modes of identification of which was only recently communicated by Major Sinton, I am practically sure of the correctness of the diagnosis of the other two forms of *Phlebotomus*. With regard to the two members of the genus *Psychoda*, the same method was adopted. Recently, however, I was given to understand in a private communication by Dr A. L. Tonnoir from Australia, who is a leading authority on the sub-family Psychodinae, that the entire group is in the melting pot. He is also of opinion that some of Brunetti's species of the sub-family are open to question. I have therefore thought it advisable to send the two specimens of the sub-family Psychodinae to Dr Tonnoir for proper identification. He has since then identified them as *Psychoda alternata* Say = (*sexpunctata* Curt.) and *Telmatoscopus albipunctatus* Willst. = (*meridionalis* Eat.)

All the drawings were made by me with the help of the camera-lucida and later on the pencil sketches were finished by Mr H. M. Roy.

* This chemical while clearing keeps the pigmentation of the sclerite more or less intact. The larva being active while alive close observation is difficult unless it is killed.

† I intend to deal with the larvae of the *minutus* group later on as time and opportunity occurs.

4 GENERAL STRUCTURE AND FUNCTION OF THE TERMINAL SEGMENTS AND THEIR MODIFICATIONS

The family Psychodidae is subdivided into two sub-families, viz, Psychodinae and Phlebotominae. Although their inter-relationship is close from the systematic point of view, yet, biologically, they are not so closely placed as one would naturally expect. The former is generally (except only one species) phytophagous, while the latter is a veritable blood-sucker. With this deviation in the habit of the imagines, the larvæ also differ considerably in their habitat. If the aquatic habitat of the larvæ, as is the case with some other groups of insects, is a factor for determining the relative archaic nature of the group, then the sub-family Psychodinae is the more primitive of the two. The blood-sucking habit of the other sub-family and the terrestrial nature of the habitat of its larva is apparently a later acquisition. Thus, the larvæ of the sub-family Psychodinae have more or less a cylindrical body in accordance with their semi-aquatic environment, while those of the sub-family Phlebotominae are somewhat flattened dorso-ventrally* in compliance with their terrestrial adaptations which is so well acquired in the sub-family as to evolve a series of 'pseudo-legs'. With this change in their adaptations, the tracheal system undergoes considerable modifications with the result that the spiracular openings change their position.

In the semi-aquatic larvæ of the genus *Psychoda*, the terminal segment becomes modified into a tubular structure tapering posteriorly with the spiracular apparatus† arranged at the tip on a circular disc. The elongated brush-like groups of hairs on the spiracular openings and on the circular disc itself, as in the case of other aquatic larvæ, apparently aid in the creation of surface tension and thus keep the tail end afloat. The anal pore is situated a little further anteriorly and mid-ventrally. It is a semi-circular or ovoidal opening with operculum-like structure or structures to close it. Besides the variations in the colour and the general shape of the terminal segment of the larvæ, specific differentiation was traced, at least in two Indian species of the genus, to the structure and number of the sclerite or sclerites of the operculum that aid in the closing up of the anal pore.

The *Phlebotomus* larvæ, on the other hand, are remarkably terrestrial and with the modification of the sclerites of the dorso-ventrally flattened terminal segment, admirably adapted as an adjunct organ of locomotion in the larvæ as well as a fixing mechanism for the pupæ, the posterior pair of stigmata becomes shifted to the penultimate segment and their external openings placed apart from each other (Plate XVIII). The terminal segment is roughly sub-quadrate with certain modifications peculiar to the species. There is nearly always a pigmented dorsal (*d s*) and an unpigmented or less pigmented ventral sclerite (*v s*) from which the

* Not exactly round in transverse section.

† 'Hinter stigma,' 'Stigmennarbe' and the 'Aussere Öffnung des Stigmensackes' of De Meijere (1915 16).

different types of spines and bristles arise. The number and nature of these spines and bristles (2 plumose, 2 spinose, 2 bristly) on each side have been found to be constant (6 in number, I, II, III, IV, V, VI, Plate XVIII), in *Phlebotomus argentipes* Ann and Biun, *P. papatassi* Scop, and *P. minutus* Rond (*sensu lato*), while specific differences have been traced in the structure of the dorsal and ventral plates as well as in the extent of pigmentation in the caudal and penultimate segments*. The number and type of the main spines on the penultimate segment are also more or less constant (4 in number, 1, 2, 3, 4, all plumose) in the three species, leaving aside, however, the small plumose spine (3) at the postero-lateral margin of the spiracle of the larvæ of *argentipes* and *papatassi*.

That the caudal segment is essentially a supplementary organ of locomotion throughout the larval life-history is proved by the existence of 2 sets of muscle insertions mesially as seen in Plate XIX. There is also a muscle disc semilunar in shape, transversely ridged and mid-ventral in position. This structure is apparently a modified sucker foot for the final fixation of the larvæ in the prepupal stage†.

Among the spines of the terminal segments, the caudal bristles, 4 in number and nearly always of shining black tint, are the most remarkable of them all. They are considerably elongated in comparison to other spines and in Nature, the inner pair of caudal bristles are held nearly vertical while the outer are horizontal. I have observed also very slow movement of these caudal bristles but whether that was due to the movement of the bristles themselves or to that of the terminal segment, I am doubtful. Most of the workers on the larval morphology have noted the characteristic features of these bristles, but, so far as the available literatures are concerned, I have failed to trace any good description of the microscopic structure of these bristles at high magnification. All that I have been able to gather is the description of Newstead (1911-12) who thinks that at high magnification a number of extremely fine, equidistant and extremely black surface lines are visible, the intervening spaces being distinctly paler. He therefore concludes that these bristles are finely striated. My observation with *P. argentipes* in this direction can be summarized as follows. I had at my disposal several mounted and stained specimens cleared in KOH. On an examination of these bristles under high power, I failed to trace any striated structure with alternate pale and dark surface markings. There was nothing very remarkable at or near the bases of these bristles, the flagellum

* So far as my knowledge goes these characteristics are traceable even in the primary stages of all the three species in question.

† A portion of the shrivelled up larval exuvium of the caudal region nearly always remains attached to the fixed terminal end of the puparium. Sometimes a close examination of this excrescence reveals the identity of the pupa. Unfortunately, identification of the larval exuvium cannot be taken as accurate, since the shrivelled up nature of the chitin sometimes misrepresents the nature of the original structure. As this work is only a preliminary contribution, I intend to deal with the pupal characteristics in the near future.

or tail end also showed no peculiarity. It is only in certain cases that an area was traced where alternate constriction and flattening out was observed forming nodes and internodes (Text-fig 1). Excepting this, the bristle is perfectly tubular and no surface markings were visible even under the high power (oil immersion). Whether this was interpreted as presenting a striated appearance by Newstead, I cannot definitely say.



Text fig 1

Microphotograph of a caudal bristle showing nodes and internodes × highly magnified

As regards the function of these peculiar bristles, I am of opinion that in addition to their functioning as an organ for scaring its enemies (parasites and predators) they probably act as tactile sensillæ of trichoid type and are thus of importance in the not unusual backward movement of the larva.

*Sub-family Psychodinae **

Genus Telmatoscopus Eat

(a) Terminal segment of the mature larva of *Telmatoscopus albipunctatus* Willst = (*meridionalis* Eat) Brun (Plate XX, fig 3)

As noted before, the terminal segment is concolorous with the body and sub-conical in shape. Elongated spines sparsely distributed cover its outer surface.

* Life history and morphology of the immature stages of individual members of the sub family have been worked out by Johnson, Faurborn, Haseman, Dell, Fullaway, Zuelzer, Efflatoun, Zavattari and a few others, but these are of importance from the biological and morphological points of view.

Three separate sclerites, triangular in shape, with their outer fringes furnished with rather thick short backwardly directed spines, apparently act simultaneously for closing up the anal pore. The two latero-ventral opercula (*lv o*) are nearly vertical in position with only one-third of their bases hinged. A portion about one-third of the anterior hinged part of these lateral sclerites presents a spotted appearance, while the rest is more or less smooth. This smooth portion bears two lateral spines. The mid-ventral operculum (*vo*) of the anal pore is hinged at its broad base with its free angular apical end directed posteriorly. The surface of this sclerite is smooth but the area near its base is similarly spotted as in the cases of the lateral sclerites.

Genus Psychoda

(b) Terminal segment of the mature larva of *Psychoda alternata* Say = (*scxpunctata* Curt.) Brun (Plate XX, fig 2)

The sub-conical caudal segment is more uniformly built than in the previous case, but is less stout. It is also externally furnished with sparsely distributed elongated spines. The semilunar anal pore is closed by a single piece of smooth operculum somewhat hemispherical in shape, mid-ventral in position (*vo*) with the anterior one-third of its base hinged. The sclerite is apparently homologous with the mid-ventral sub-triangular operculum of the previous case.

Genus Phlebotomus

(c) The two terminal segments of the mature larvæ of *Phlebotomus argentipes* Ann and Brun (Plate XVIII)

The sepia-tinted pigmented area of the penultimate segment begins on the dorsum at the region where the pair of hairs (I) originates. The pigmented portion extends anteriorly to more than one-half of the entire area of the segment. More than half of the distal region of the dorsum of the caudal segment including the dorsal sclerite and the protuberances from which the four caudal bristles originate is tinted dark. As the pigmented area extends anteriorly the colour becomes paler until the distal border of the penultimate segment is reached. Posteriorly, a thin strip of the ventral sclerite is also pigmented. In the young larvæ the pigmentation approaches to some extent that of the mature one. The caudal segment with its appendages is roughly sub-quadrate in shape. The dorsal sclerite (*ds*) is horse-shoe shaped, smooth dorsally and is somewhat smaller than the ventral sclerite when viewed dorsally. A convexity bearing denticles marginally gives the characteristic horse-shoe shaped appearance. The dorsal sclerite occupies nearly half the entire area of the segment. The two short stout arms are broadly rounded off and bears at each of their outer angles a short plumose spine (I). A little below these arms the protuberances (*pn*) bearing the four bristles, two on each protuberance (II and III), are situated. The ventral sclerite (*vs*) is roughly quadrate in shape, distinctly paler in contrast to the dorsal sclerite, and with

its distal margin almost straight. Each of its angular regions bear outwardly three equidistant spines (IV, V, and VI). The IVth being the longest, the Vth plumose but larger than the Ist while the VIth is equally developed as the IVth. The ventral spines of the caudal segment not shown in the figure are six in number—3 on each side and arranged in an arc. These spines are all simple and pointed, the lateral ones being nearly double the length and thickness of the two inner ones.

(d) The terminal segments of the mature larva of *Phlebotomus papatassi* Scop. (Plate XIX)

In contrast to *P. argentipes*, the terminal segment is well demarcated off in *P. papatassi*. The pigmented area of light sepia tint of the penultimate segment covers more than half the entire area of the segment*. In the caudal segment the pigmentation of the dorsum is somewhat darker at its distal margin including the two protuberances from which the four caudal bristles originate. The dark sepia-tinted area of the dorsal sclerite covers only about one-fourth the entire area of the segment which is comparatively more elongated than in the case of *P. argentipes*. This pigmentation extends latero-marginally forwards covering the edges of the dorsal sclerites to about three-fourth the whole length of the segment. The pigmentation of the dorsal sclerite is somewhat paler mid-dorsally. The pigmentation in the young larva simulate to some extent the characteristic type. The dorsal sclerite in this case is more elongated and marginally extended beyond the distal border of the ventral sclerite in contrast to that of *P. argentipes*. The whole dorsum of the dorsal sclerite is more or less uniformly covered with fine spines. The dorsal sclerite is also roughly horse-shoe shaped posteriorly as in the case of *P. argentipes* but with its free arms somewhat more pointed and elongated. A similar mid-marginal concavity bearing a series of denticles is also present but in this case it is more marked. The two lateral borders of the dorsal sclerite in contrast to *P. argentipes* bear on each side concavities giving rise to the characteristic star-shaped appearance. The plumose spine (I) is larger in comparison to that of *P. argentipes*. The two protuberances bearing the four bristles—2 on each side (II and III)—as well as the bristles themselves are of no particular importance except that they are stout.

The ventral sclerite is roughly shield-shaped with its lateral margins more or less parallel to each other. The spine (IV) is well developed, curved, inwardly, the Vth is fine and not so spread out distally as in the case of *P. argentipes*, while the VIth is similar to the IVth. The smaller ventral spines of the sclerite, not shown in the figure, are as follows. Two small spines from the two distal angles of the anal pore and two somewhat larger lateral spines.

* Whittingham and Rook (1922-23) describes the pigmentation as follows. Dark brown pigmentation of the whole dorsum of the last body segment and the central portion of the penultimate segment.

(c) The two terminal segments of the mature larva of *Phlebotomus minutus* Rond (*sensu lato*) (Plate XX, fig 1)

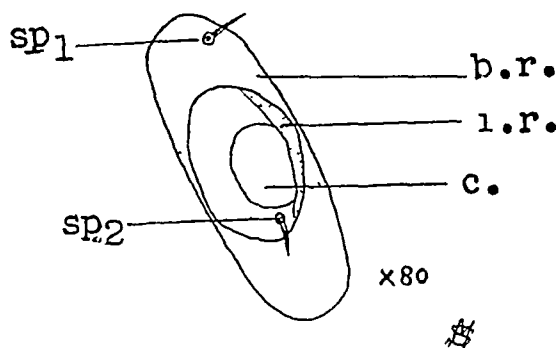
In the *P. minutus* group the two terminal segments are broadened considerably in contrast to other segments of the body. This broadening is carried so far in the group as to produce lateral projections and tubercles. Thus, we see that in this case the posterior pair of stigmata assume a complete lateral position along with the lateral hairs which seem to originate from lateral tubercles. The penultimate segment thus assumes the shape of the multi-tuberculated head of a club. Nearly the whole of the penultimate segment is light brown in colour. The dorsal plumose spines of the *minutus* group show some characteristic differences both as regards their number and shape in contrast to *P. argentipes* and *P. papatassu*. Spine (1) is reduced to small stumpy size, spine (2) assumes a lateral position, spine (3) is untraceable, while spine (4) takes a position just ventral to the spiracle. The caudal segment is distinctly demarcated off from the penultimate segment and is roughly heart-shaped. It is characteristically pigmented in contrast to the other two members of the erect-haired group. The peculiar pigmentation of the dorsum of the dorsal sclerite is due to the presence in the anterior two-thirds of the segment of a semilunar, pale, bare, apparently depressed area with its broad base reaching the distal margin of the penultimate segment. The distal margin of the dorsal sclerite bears the usual dentated concavity. The semilunar portion of the dorsal sclerite including the two protuberances bearing the four caudal bristles are characteristically pigmented dark brown dorso-laterally and its surface furnished with fine spines. Mid-dorsally it is almost black. Plumose spine (I) is comparatively well developed and longer, caudal bristles (II and III) are of no particular importance. The ventral sclerite of the caudal segment developed quite in conformity with dorsal sclerite so that no portion was visible from the dorsal aspect. Spines (IV, V, VI) deserve no particular mentioning. Ventrally the ventral sclerite bears four fine spines arranged in an arc and two larger spines placed laterally.

5 A SHORT COMPARATIVE ACCOUNT OF THE MICROSCOPIC STRUCTURE OF THE 'PSEUDO-LEG' OF *Phlebotomus argentipes* ANN AND BRUN (Text-fig 2)

The special locomotory organs are apparently a later acquirement of the *Phlebotomus* larvæ and homologous with similar structures of the Lepidoptera larvæ, but they are devoid of the typical crochets. As early as 1907, these structures were noted and figured by Grassi and subsequently by other workers*. So far as I could gather, the species dealt with in this way was exotic. It therefore devolved on me to record my observations on an Indian form more or less on a comparative basis.

* Whittingham and Rook (1923) figures the false legs of *P. papatassu* on all the segments except the first three. He also opines that the filamentous hairs and the sucker-like false legs give them firm foothold.

In the figure of 'Dischi succhiatori' (suctorial disc) of *Phlebotomus papatasi* by Grassi, the outline of the disc is more ovoidal than that of *P. argentipes* where it is flatly ellipsoidal in optical section. To differentiate the separate concentric structures of this compound organ, I could not but adopt several non-committal names. Thus the slightly raised chitinous portion with a fine pointed spine (sp_2), eccentrically placed near the margin, has been named basal rim (br). The inner rim (ir) is saucer-shaped in optical section and is the real suctorial structure, it attains a slightly higher level than the basal rim. The inner rim bears also an eccentrically placed similar spine (sp_1). The deeper part of the inner rim bears a small concavity (c), apparently the area of insertion of a strong set of muscle which by contraction creates a vacuum and thus help in the looping movement of the larva both on horizontal and inclined planes.



Text fig 2

Microscopic structure of a pseudo leg

Grassi's figure of 'Dischi succhiatori' shows two plumose (?) elongated spines arranged symmetrically on the two lateral poles of the third concentric rim. I have, however, failed to trace any other spine excepting the two small pointed eccentrically placed spines mentioned above.

6 SUMMARY

1 The observation of previous workers on this line have been critically reviewed. It is pointed out that the study of the larvae of the family Psychodidae from the systematic point of view is not on a par with that of the imagoes.

2 The technique employed and considered most suitable for this type of study has been described.

3 The general structure and functional importance of the terminal segments in Psychodidae larvae are described in detail. It is concluded that these structures are of considerable importance throughout the larval life-history and even during the primary stage of pupation. It is further noted that the semi-aquatic-type of Psychoda and Telmatoscopus larvae is presumably the more archaic form while the terrestrial type of Phlebotomus larvae is only a later acquisition in response to

changing environment and habit. This is perhaps to some extent borne out by the fact that the *Phlebotomus* larvæ require a humid atmosphere for the completion of their life-history.

4. A comparative study of the terminal segments of two species belonging to the genera *Psychoda* and *Telmatoscopus* and three species of the genus *Phlebotomus* is given. It is suggested that the terminal segments might play an important rôle in the classification of the larvæ of the family Psychodidae.

5. A short comparative note on the microscopic structure of the pseudo-legs of *Phlebotomus argentipes* Ann and Brun is appended.

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EXPLANATION OF LETTERING IN PLATES XVIII TO XX

<i>b r.</i>	Basal rim of the pseudo-leg
<i>c</i>	Concavity where vacuum is produced by strong set of muscle inserted dorsally
<i>d s</i>	Dorsal sclerite of the caudal segment
<i>i i</i>	Inner rim of the pseudo-leg
<i>lv o</i>	Latero-ventral operculum
<i>pr</i>	Protuberance bearing the paired caudal bristles
<i>sp₁, sp₂</i>	Spines on basal and inner rims of the pseudo-leg
<i>St</i>	Stigmatas (posterior)
<i>v o</i>	Ventral operculum
<i>v s</i>	Ventral sclerite of the caudal segment
I, II, III, IV, V, VI	Hairs of caudal segment (visible from dorsal aspect)
1, 2, 3, 4	Hairs of the penultimate segment (visible from dorsal aspect)

PLATE XVIII

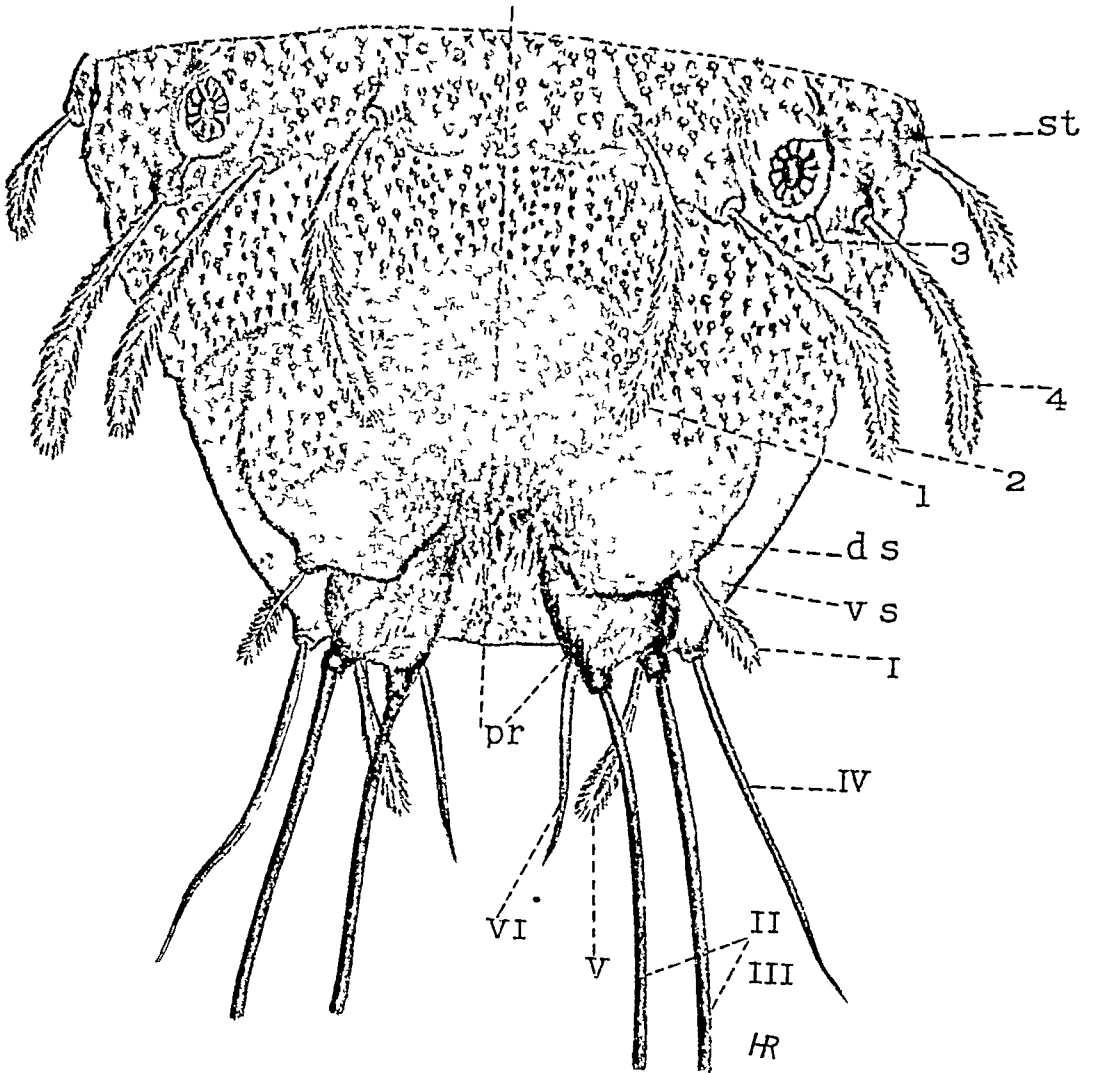
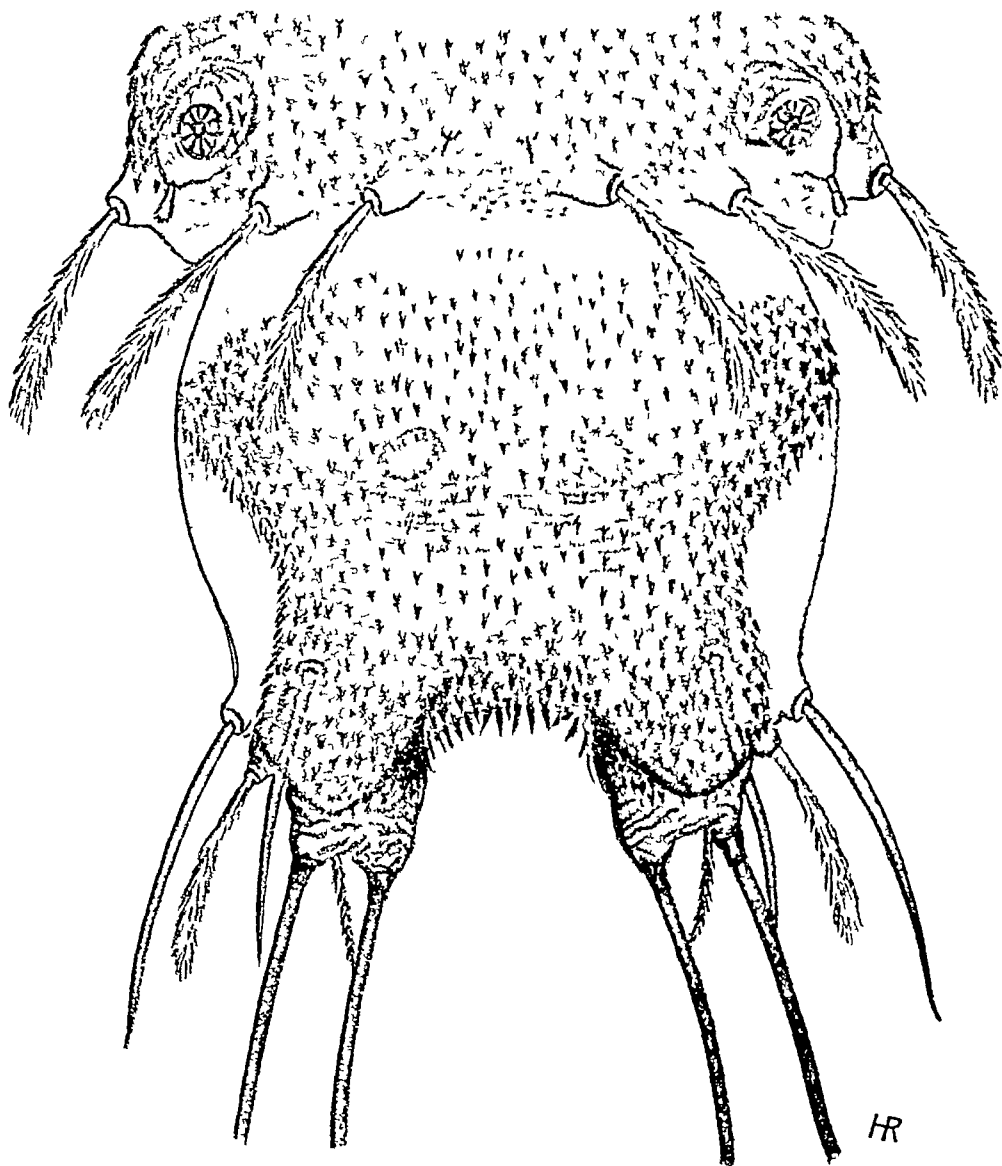
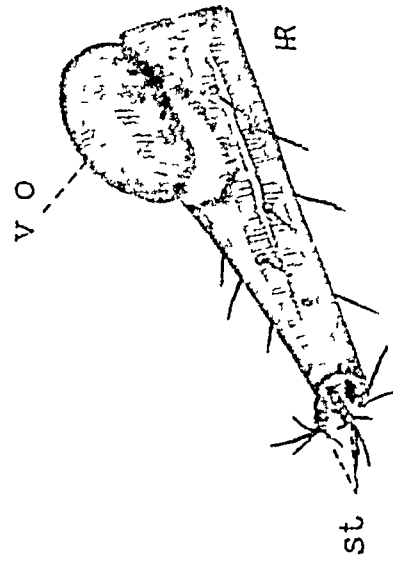
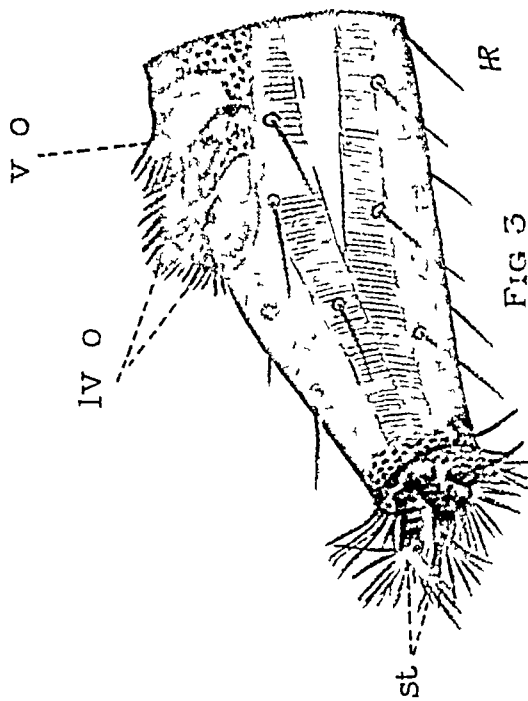
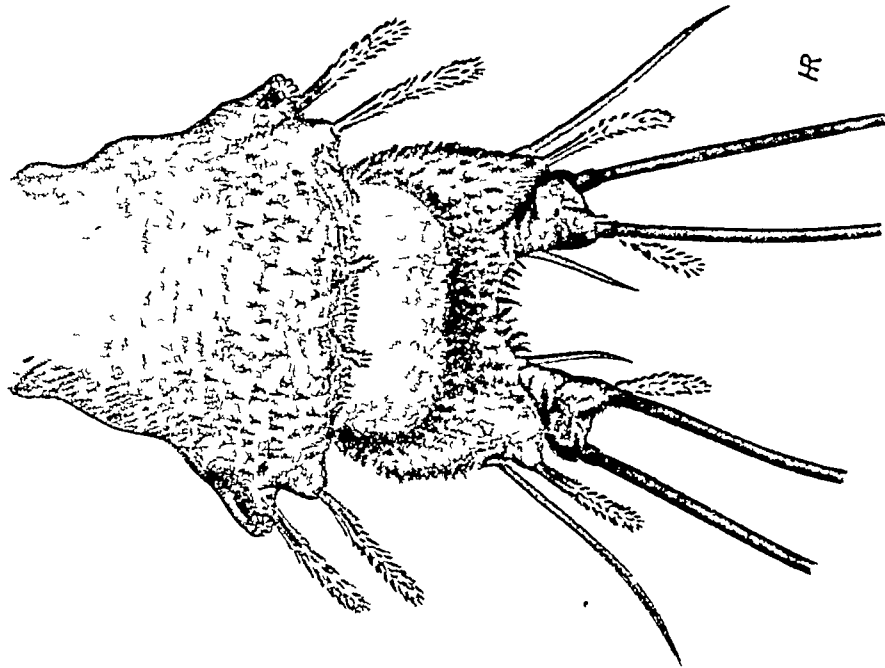


PLATE XIX





DEEP TUBE-WELL WATERS OF BENGAL.

BY

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[Received for publication, April 27, 1931]

WITHIN the last ten years deep tube-wells have become a favourite source of drinking water supply in various parts of Bengal and are continuing to fulfil a long-felt want for pure drinking water. It has been clearly established that an abundant quantity of water is stored up below the surface in this part of India and that this underground water is under such great pressure that even in the case of tube-wells of 300 to 400 feet deep the water rises to within 20 to 30 feet of the surface. The presence of a large amount of water under sub-artesian conditions is an asset of great value from both the agricultural and the public-health points of view. Further, these waters possess certain interesting chemical and bacteriological characteristics and are therefore of more than mere parochial interest. The writer also hopes that with further knowledge it may be found that underground water is also similarly present in at least some other parts of India, where conditions may be favourable for storage under sub-artesian conditions such as an abundant rainfall in neighbouring hills and an impervious layer below the surface.

Samples from over 150 tube-wells have been examined on one or more occasions. In the case of 23 tube-wells systematic monthly examinations were made for a period of two years. Daily samples were also examined from six tube-wells for a period of three months beginning from before the onset of the rains.

BACTERIOLOGICAL RESULTS

The bacterial contents of these tube-well waters are in marked contrast to those of surface waters, both in the number and the variety of the organisms met with. Of the 500 monthly samples examined 51 per cent failed to show lactose fermenters in 60 c.c. and 68 per cent showed a negative result in 10 c.c. Only

15 per cent of the samples showed a positive result in 1 c c. A study of the types of lactose-fermenting organisms in the above monthly examinations is also interesting. Out of over 1 600 organisms examined, only 19 per cent belonged to the typical *B. coli* type (Indol + V P—). This is in marked contrast to the state of affairs in surface waters where 75 per cent may belong to the typical *B. coli* type. The 19 per cent of typical *B. coli* found in the tube-well waters comprised the following species —

<i>B. coli communis</i>	4 per cent
<i>B. neapolitanus</i>	6 „
<i>B. coscoroba</i>	3 „
<i>B. vesiculosus</i>	5 „
<i>B. acidilactici</i>	1 „

The remaining 81 per cent belonged to the *Cloacæ ærogenes* type.

The results of the daily examinations of tube-well waters have also been very interesting. Of the six tube-wells thus examined, one only showed a uniformly good bacteriological result. Out of 73 samples examined from the latter all gave a negative in 60 c c. The total bacterial count on nutrient agar was none in one cubic centimetre in more than a third of the samples examined and was, except in four cases, as low as 1 or 2 per cubic centimetre. It is evident that the subsoil water has attained a high degree of bacterial purity. The remaining five tube-wells have, however, not showed such a favourable result as the above-mentioned one which was newly constructed. From among the five tube-wells the number of samples showing a positive result for lactose fermenters in 10 c c. was 23, 26, 20, 49 and 33 respectively (73 samples were examined in each case). In all cases some were positive in 1 c c. Their actual number was 3, 6, 9, 27 and 10 respectively. The number of samples showing a negative result in 60 c c. was 27, 34, 31, 9 and 23 respectively. Tube-well No. IV has shown the worst result with 49 samples positive in 10 c c., 27 positive in 1 c c. and only 9 samples negative in 60 c c. The types of lactose fermenters isolated is also significant. 81 per cent were indol negative and belonged to the *Cloacæ ærogenes* type and only 19 per cent were indol positive and typical *B. coli*.

It is noteworthy that even tube-wells situated within 3 or 4 feet of each other and similarly and simultaneously constructed should have shown widely different degrees of bacterial purity. Rainfall may be ruled out altogether as a causative factor as no relation whatever could be detected between it and the variations in the bacterial content. The presence of bacteria seems to have some relation to the corrosiveness of the water, as waters exhibiting this property markedly have shown the worst result bacteriologically.

The question arises, where do these organisms come from and what does their presence signify. There is considerable evidence to justify the conclusion that

they undoubtedly are not the effect of faecal contamination. In the compact clayey soil surrounding the tube-well there can be no percolation to any marked extent. Even if it were present to a small extent it should be more marked in newly constructed tube-wells than in old ones. Actually, however, the reverse has been found. Secondly, the total bacterial count has generally been remarkably low which would not be the case had there been faecal pollution to any extent. Thirdly, the chemical results do not give any evidence of an admixture of surface water with the sub-soil water. Although the tube-wells have in many cases been located within a few yards of the bed of a river the results of chemical examination of the tube-well water were very different from those of the river water. The marked seasonal changes in the chemical characteristics of the river water were not at all reflected in the quality of the tube-well water. As a matter of fact a slight negative correlation was observed between the two. When at the height of the rains the river water showed its lowest figure for salinity and hardness, the tube-well water showed an actual increase in the above contents. There can therefore be no possibility of any passage of surface polluted water into the tube-well. Fourthly, the results of chlorination of the tube-wells are very significant. A strong solution of bleaching powder was let into the tube-well and allowed to remain for some hours and then pumped out. This resulted in the lactose fermenters being entirely eliminated and they did not show their appearance again till another fortnight had elapsed. The beneficial effect of chlorination could not last for so many days if there was systematic passage of polluted water into the tube-well. One is therefore forced to the conclusion that these organisms were not derived from faecal pollution, but are the result of multiplication at some favourable position, such as a leaky joint. The tube-well waters are highly corrosive and big holes in the tubes have actually been observed when some tube-wells were detached.

The above conclusion that lactose fermenters are able to multiply in nature under certain natural conditions is one of great importance from the point of view of bacteriological standards. It is usually, and rightly so in most cases, assumed that these organisms do not multiply outside the animal or human intestine. If it were not so, their numbers would not serve as an index of the degree of faecal contamination. While, undoubtedly, it is true that they do not generally multiply outside their natural habitat in the animal or human intestine, still it has to be recognized that they occasionally do so under certain conditions. In a previous communication (Govinda Raju, 1922) the author pointed out that the lactose fermenters did increase in numbers in under-ground reservoirs when the water was stagnant for some hours. In old pipes also they seem to find conditions favourable for a slight increase in numbers.

The above conclusion that *B. coli* or organisms indistinguishable from them may occasionally be found in localities free from faecal pollution has also been

reached by some other investigators. In the Philippine Islands Otto Schobl and José Ramirez (1925) of the Biological Laboratory, Bureau of Science, Manila, found that the source of lactose fermenters found in their artesian wells was not faecal contamination at all but that the organisms were derived from some parts of the well itself. When these parts and the inside of the well were disinfected the water became sterile indicating clearly that the organisms were not the result of faecal contamination, but due to multiplication. Further, new and unused packing and washers, which up to the time of bacteriological examination had not been handled (having been delivered for examination as originally wrapped), were also found to contain lactose fermenters. They appear to have been mostly of the *Cloacaeiogenes* type. Sir Alexander Houston (1916) found in the leather washers of taps organisms similar to *B. lactis eiogenes* and fermenting indol. This was called the leather bacillus and after a detailed study he concluded that the current quality of a tap-water sample could not be judged by the presence of bacteria, answering the characteristics of the leather bacillus whose origin was in the leather washer of the tap itself.

CHEMICAL RESULTS

The results of chemical examination of tube-well waters show several interesting features. The first point that arrests attention is the variation in the salinity of the water. It has no relation with the bacterial quality of the water and is quite independent of faecal or urinary contamination. A scrutiny of the figures shows that the salinity (expressed as chlorine) is remarkably low (1 to 2 parts per 100,000) except in close proximity to areas where tidal rivers are flowing at present or have flowed in past times. In the Howrah district where tidal influence is fully felt in the river the salinity of the tube-well water varied inversely with the distance of the tube-well from the river. In areas close to the river the salinity was invariably very high and ranged from 60 parts to even 150 parts per 100,000 (expressed as chlorine). The salinity decreases as we proceed inland until it is only 2 to 3 parts in areas about fifteen miles inland. Similarly, in the 24-Perganas district where also tidal influence is felt in the river, the salinity of the tube-well waters followed more or less the salinity of the adjoining river water. Above Shamnagar about 20 miles up the stream from Calcutta both the tube-well water and river water showed a salinity of only 1 part per 100,000 and in both cases the salinity steadily increased as we proceeded lower down. Adjoining the areas where the river is permanently saline, the tube-wells also showed very high salinity. This parallelism between the salinity of the tube-well water and that of the adjoining river water does not, however, seem to be due to the direct admixture of river water into the tube-well but to the impregnation with salt of the soil surrounding the tube-well. This is

evident from the fact that while the salinity of the river water varied at different seasons of the year and even hourly at different times of the tide, the salinity of the tube-well water remained practically constant. Even when there was some variation in the salinity of the tube-well water it varied inversely with that of the river water, indicating that there can be no direct connection between the two.

It is an anomalous fact that the lowest figure for salinity and hardness was furnished by a tube-well adjoining the sea-coast. Obviously, the source of the water in this case is the rainfall in the neighbouring hills within a few miles off. The low figure of 0.6 part per 100,000 for salinity shows that the water could not have traversed any great distance. In areas not adjoining tidal rivers or tidal swamps the figure for chlorine was usually one to two parts per 100,000. Rajshahi, however, 150 miles inland showed 4 parts. It is highly probable that in past times the tidal influence in the river was felt in this part also. Some writings in the eighteenth century furnish evidence that at about that time the river began to silt up. The salinity of the tube-wells sunk in or near Calcutta showed wide variations. The lowest figure was 2 parts and the highest 66 parts, expressed as chlorine. It is noteworthy that excessive figures for salinity were obtained even at some distance from the adjoining tidal river. The explanation of this is that although the Hoogly now flows to the west of Calcutta, there is to the east of Calcutta a highly saline swamp which was formerly a tidal creek. Thus Calcutta is practically surrounded in most places by salt-impregnated soil, only a small area being comparatively free from saline matter.

HARDNESS OF TUBE-WELL WATERS

The hardness, or the soap-destroying power, of these waters, is generally high. The only exception so far met with is the water of the tube-wells in Chittagong, which also showed the lowest figure for salinity. At Chittagong the temporary hardness was 3 parts and the permanent hardness 2 parts per 100,000. In no other case have the figures been so low as in the case of the Chittagong tube-wells. The tube-wells in Burdwan, Dacca and Mymensingh districts have also yielded a fairly soft water. In Burdwan the temporary hardness was 3 parts and the permanent hardness 4 parts. In Dacca and Mymensingh the temporary hardness was about 10 parts, and the permanent varied from 2 to 4 parts. The interest of the above observation lies in the fact that in these places the river water also is much softer than elsewhere in Bengal and gives for chlorine and temporary hardness figures more or less similar to those for the tube-well water. The permanent hardness of the latter is, however, much greater. Usually the permanent hardness of tube-well waters varies from 3 to 6 parts which figure may be looked upon as normal even in areas away from tidal rivers. Generally speaking, the temporary hardness was never less than 20 parts. About 20 to 25 parts may be taken as the rule for tube-well waters in areas not adjoining tidal rivers. In the latter case the

temporary hardness is higher and may reach 40 parts. The permanent hardness is also much higher and may vary from 50 to 100 parts. In these tidal areas the permanent hardness is much higher than the temporary hardness while ordinarily in surface waters the temporary hardness is invariably higher than the permanent hardness. The high permanent hardness is generally associated with high chlorides.

Seasonal variation in hardness and chlorides were formerly not believed to occur in tube-well waters. To settle this point systematic daily examination from six tube-wells was carried on for a period of three months during a time of the year when changes are most likely to occur. It was found that some variation does take place and that these changes are the reverse of the changes taking place in the adjacent river waters. In the case of the surface waters the hardness and chlorides are considerably reduced after the establishment of the monsoon rains and this reduction may be so great as to reduce the total hardness to a fourth of what it was a month previously. While in May it may reach 25 parts it may fall to 6 parts in June. Similarly the chlorides are also considerably reduced in the case of river waters. On the other hand in the case of tube-well waters there is a slight increase in both the hardness and chlorides after the outburst of the monsoon. This increase, however, is only slight and may be due to the general raising of the underground water during the monsoon and the consequent admixture of some saline water with the body of water feeding the tube-well. The following table shows the actual figures obtained —

	Before rains	After rains
Chlorine	1.0 part per 100,000	1.2 parts per 100,000
Temporary hardness	11.0 parts „	13.0 „ „
Permanent hardness	4.2	6.2 ,

AMMONIAS AND ORGANIC MATTER

The figures for albuminoid ammonia and the permanganate test were almost invariably very low indicating that there was practically no organic matter in the water. Apparently in its underground passage through miles of sand the entire organic matter is strained out or oxidized. A similar straining action has been observed in the case of slow sand filters which have been newly replenished with sand. The figure for albuminoid ammonia is seldom above 0.005 part per 100,000 and the figure for four hours permanganate test may be 0.025 and frequently even much less than the above figures. Occasionally the permanganate test has yielded slightly higher figures than the above due to the absorption of the oxygen by the

ferrous salts present in the water. No correction is usually made for this but seldom have the results been high enough to demand particular attention being paid to them.

The free ammonia-content of these tube-well waters demands special consideration. Contrary to the finding in surface waters the free ammonia is almost invariably higher than the albuminoid ammonia. As it is not, however, accompanied by a high figure for albuminoid ammonia or organic matter, faecal or urinary pollution may altogether be ruled out. In many cases the amount of free ammonia was very high and in several instances it has reached the excessively high figure of 0.1 part per 100,000. A very careful scrutiny of the results was made to discover if the excessive free ammonia could be correlated with any other characteristic of the water. This was not found to be possible. In some of these cases the water is soft, in others it is hard. In some iron is present in considerable amount, but in others iron is totally absent. In some nitrates are present, while in others no trace of nitrates could be found. Bacteriologically some of these are exceedingly pure, while others are indifferently so. It does not seem possible to discover an association between the excessive amount of free ammonia present and any other character of the water. A very significant observation has been made in the case of some tube-well waters which generally showed a high figure for free ammonia. Occasionally while the figures for most other constituents generally remained unaltered, the figure for free ammonia alone underwent a marked reduction and was accompanied by a marked increase in the amount of nitrates. This negative correlation between the nitrates and the free ammonia suggests the possibility that the excessive amount of free ammonia may really have been derived from the nitrates present in the water. The latter would tend to become reduced through the reducing action of ferrous salts present in the ferruginous sands through which the water has passed. This reduction may also have been effected by the material of the iron pipes. This latter is highly probable as the tube-well waters have been found to be highly corrosive to iron pipes.

Another very striking fact is the extremely low figure obtained for nitrates in most cases. Out of several hundreds of samples examined hardly a dozen samples showed nitrates above 0.05 part per 100,000. This is in marked contrast to the results obtained in European countries. The explanation may be that the nitrifying organisms do not flourish in the great depths in which the waters are flowing. The extreme bacterial purity of these waters would indicate that these organisms, even if present originally, would have been strained out as the water passes through miles of sand. Iron is occasionally present in undesirable amounts, but more often it is either totally absent or only present in small amounts which is easily precipitated, when the water is left standing overnight. Manganese has so far not been found at all in any case. The carbon dioxide content of tube-well waters is strikingly different from that of surface waters. In the

latter case there may be no carbon dioxide at all during the greater part of the year, the water being actually alkaline to phenolphthalein. During the rains, however, a small amount of carbon dioxide, 0.4 to 0.5 part per 100,000, may be present. On the other hand, the tube-well waters showed large amounts of carbon dioxide throughout the year. Almost all the waters showed above 2 parts per 100,000 and half the number of samples examined showed above 5.0 parts, while one sample showed the extremely high figure of 12.0 parts per 100,000. This excessive amount of carbon dioxide appears to confer on the water its characteristic ferro-solvent property. While the water is rich in carbon dioxide it is frequently poor in oxygen and many samples have been found to contain only a trace of dissolved oxygen.

SUMMARY AND CONCLUSIONS

(1) The underground water tapped by the deep tube-wells in Bengal appears to be practically sterile, but as actually drawn a variable number of lactose fermenters may not infrequently be obtained. This may be due to the highly corrosive nature of the water and consequent holes in the pipes leading to a multiplication of certain types of lactose fermenters. 80 per cent of the latter belonged to the *Cloaca aerogenes* type and 20 per cent to the typical *B. coli* type (indol positive and Voges and Proskauer negative). The above observation is important from the point of view of interpretation of results of bacteriological examination of water. Failure to recognize the above findings may result in ascribing the numerical presence of these lactose fermenters to faecal pollution. This occasional lack of sanitary significance of these lactose fermenters has already been pointed out by Sir Alexander Houston in the case of the leather bacillus in tap-waters in London and by Otto Schobl and José Ramirez in the case of tube-well waters in the Philippines.

(2) Chemically the tube-well waters showed certain well-defined characteristics. Outside areas adjoining tidal rivers or tidal swamps the salinity is generally very low and did not exceed 2 parts per 100,000 (as chlorine). The hardness, both temporary and permanent, is usually high. The permanent is 4 or 5 parts and the temporary seldom below 20 parts per 100,000. Free ammonia may sometimes be present in considerable excess and is generally present in larger amount than the albuminoid. As judged by the permanganate test the amount of organic matter is practically negligible. Only a trace of nitrate is generally found and iron is frequently found even in large amounts but may also be totally or practically absent.

(3) It is highly desirable to locate these tube-wells as far away as possible from a tidal river or tidal swamps.

(4) The depth of the tube-wells does not appear to be in itself an important factor as even within a depth about 100 feet a highly satisfactory supply has been obtained in several instances.

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STUDIES IN THE HISTOLOGY OF THE SPLEEN, BONE,
MARROW AND LIVER IN CASES WITH SPLENOMEGALY,
WITH SPECIAL REFERENCE TO THOSE DUE
TO KALA-AZAR

(1) THE CONNECTIVE TISSUE AND RETICULUM

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[Received for publication, April 27 1931]

INTRODUCTION

THESE studies have been undertaken for the observation of any pathological alterations which might present themselves in cases encountered here in which the spleen was enlarged, whether such changes involved tissues or various types of cells. In the necropsies performed at the Medical College Hospital during the past nine years the majority of the cases in which the spleen has been found to be greatly enlarged have been due to infection with *Leishmania donovani*. In so far as this disease is concerned the distribution of the parasites was fully and admirably described by Christophers (1904). Further contributions have been made by Laveran (1917), Meleney, Shortt (1923), Perry, De (1927), and Cash and Hu. From these studies it has been shown that the parasites could be found in any part of the body where cells of mononuclear type might be located. Their distribution in fact was that of the so-called reticulo-endothelial system. Pittaluga (1927) refers to this widespread distribution as constituting an actual pathological

'blockage' such as is produced experimentally by the injection of certain dyes and colloidal materials and this view is undoubtedly supported by the appearances observed by us

We have undertaken in this work to study the mesenchymal reactions and the alterations in hæmatopoiesis, which might result from, or be associated with, this extensive parasitization of such cells

With the studies of those cases in which the splenomegaly was due to infection with *Leishmania donovani*, we have included a number of others, in which no such etiological agents were found

In this first publication we record the results of our observations on the connective tissue and reticulum of the livers and spleens of a series of cases most of which were kala-azar infections

TECHNIQUE

The material secured has been fixed in Zenker's fluid either with acetic acid, or with formol neutralized with magnesium carbonate

The stains used have been Heidenham's Azan-Färbung (1928) for fibrous tissue—a modification of Mallory's aniline blue method, and Foot's modification of the Bielschowsky-Maresch technique for reticulum

By these methods we have studied a series of thirty-one cases in twenty-five of which we demonstrated the presence of *Leishmania donovani*

In a twenty-sixth case a culture of flagellates had been secured but *Leishmania donovani* could not be discovered post-mortem

Notes on these cases and on the histological appearances are presented in the following table —

It will be seen from the table that, in twenty-three cases in which *Leishmania donovani* was demonstrated no increase of fibrous tissue or of reticulum occurred in the spleen. In two cases there was slight coarsening of trabeculæ and reticulum fibrils

In the twenty-sixth case (No. 1) in which a flagellate culture had been secured before death but in which *Leishmania donovani* was not demonstrated post-mortem there was slight apparent increase of reticulum in the spleen. In this case however there was a complication in the existence of widespread tuberculosis including the presence of tubercles in the spleen

Of five cases not due to kala-azar, three showed slight coarsening and increase of reticulum fibrils

In the liver in one case (No. 19) there was a definite, marked cirrhosis of the liver chiefly of the Lænnec type

In five cases (Nos. 13, 18, 24, 29 and 31) there was slight portal fibrosis, which was not, however, widely spread. In three cases (Nos. 5, 11 and 12) there was slight patchy fibrosis

TABLE

No	No of necropsy	Age	Race	Sex	Clinical note	Anatomical diagnosis at necropsy	L D B in sections	SPLEEN			LIVER		
								Weight and gross appearance	Heidenhain's stain	Foot-Belschowsky stain	Weight and gross appearance	Heidenhain's stain	Foot-Belschowsky stain
1	42-26	24	Hindu	Male	Fever Anemia (1,250,000 red cells per c mm Hemoglobin 30 per cent) Spleen enlarged Flage llate culture—positive	Enlarged fibrous spleen, hydro peritonium, caseous lymphadenitis about pancreas and liver, bilateral hydrothorax, milary tuberculous, right lung and pleura, pleuropleurisy—right side, hydropericardium dilated right ventricle and atrium Necropsy eight hours post mortem	0	760 gm Large, firm Dark red	No thickening of capsule Trabeculae not thick A fair amount of collagen about arterial walls Tubercles present	Amount of reticulum somewhat increased—Fibrils marked about and in the tubercles	1,230 gm firm Congested	No increase of collagen.	No increase of reticulum
2	48-26	40	European	Male	Anemia (Hemoglobin RBC 1,400,000 WBC 2,500), Wassermann and Aldehyde reaction negative Antimony treatment for kala azar in 1922 in hospital	Fat and fibrotic liver, esophageal varices with rupture and haemorrhage enlarged spleen calcification thyroid cartilage ascites Necropsy 16 hours post mortem	0	1,290 gm Capsule thick and fibrous Firm Colour dull red	Fibrosis about central arterial Traculles fairly marked	Fibrils in pulp not conspicuously increased in amount or thickness	1,320 gm Pale, finely nodular and contracted	Slight patchy portal cirrhosis	"

TABLE—contd

No	No of necropsy	Age	Race	Sex	Clinical note	Anatomical diagnosis at necropsy	L D B in sections	SPLEEN			LIVER		
								Weight and gross appearance	Heidenhain's stain	Foot Bielschowsky stain	Weight and gross appearance	Heidenhain's stain	Foot-Bielschowsky stain
3	67-26	16	Hindu	Male	Anemia RBC 1,190,000, WBC 3,500 Aldehydic reaction negative Normoblasts present	Enlarged spleen, fatty liver, bilateral hypostatic pneumonia Large white kidneys Ankylostomiasis Necropsy nine hours post-mortem	0	720 gm Perisplenic fibrous looking Two accessory spleens present	Trabeculae thick Some arterioles show irregular intimal fibrosis No general fibrosis however	Fibris not definitely increased in amount of thickness	1,680 gm Firm, pale and fatty looking	In some portal areas collagen slightly increased No actual cirrhosis	No increase of reticulum
4	75-26	32	Hindu	Male	Diagnosis Pulmonary phthisis with laryngitis	Pulmonary tuberculosis, bilateral pleurisy, enlarged liver and spleen, acute nephritis, dilatation right ventricle, tuberculous ulceration of larynx, trachea, and large intestines, cholelithiasis Necropsy twenty two hours post mortem.	+	1,470 gm Fairly firm Colour dull red Splenic vein much dilated	Fibris and trabecular bands not increased	Fibris not definitely increased	2,370 gm Congested and appears moderately fatty Firm on section	No increase of fibrous tissue in portal areas	"
5	52-27	32	Moham median	Male	Clinical diagnosis Kala-azar Aldehydic reaction—strongly positive—no tubercle bacilli in sputum Urea stibamine given	Enlargement of spleen and liver empyema thoracis sinistra, purulent pericarditis Necropsy seven hours post-mortem	+	1,290 gm Soft and almost diffuent	Capsule not thick Trabeculae few No increased amount of collagen noted anywhere	Reticulum fine fibris and not increased in proportion to other structures	2,370 gm Firm and showing chronic passive congestion	There is a diffuse increase in collagen	There is a moderate diffuse increase in reticulum

6	54-27	55	Anglo Indian	Male	Nephritis was the main diagnosis	Enlargement of spleen and perisplenitis, chronic nephritis, anasarca, atheroma of aorta Necropsy six hours post-mortem	+	450 gm Early soft	Trabeculae fine fibrous	Retioulum fibrils fine and of average proportion	1,020 gm Firm Surface granular per hepatitis	No increase of fibrous tissue anywhere	No increase of reticulum.
7	56-27	20	Hindu	Male	Diagnosis Kala-azar with hepatitis History of twelve months' fever	Enlargement of spleen and liver, cholelithiasis, plastic pleurisy right side enlarged thymus Necropsy fourteen hours post-mortem	+	1,380 gm Soft Dull red in colour	No increase of fibrous tissue	No increase of reticulum fibrils	2,100 gm Enlarged, soft	No increase of fibrous tissue anywhere	"
8	59-27	28	Hindu	Female	Diagnosis Pulmonary phthisis—cough and fever of two months' duration	Enlargement of spleen, plastic pleurisy—bilateral, perisplenitis Necropsy twenty hours post-mortem	+	570 gm Firm Dark red in colour	No increase of fibrous tissue	No increase of reticulum	132 gm Early soft in texture	No increase of fibrous tissue anywhere	"
9	75-27	22	Hindu	Male	Diagnosis Kala-azar, Pneumonia Nephritis	Enlargement and congestion of spleen and liver, pneumonic consolidation of apex upper lobe and of lower lobe right lung, large pale fatty kidney, Necropsy five hours post-mortem	++	1,170 gm Perisplenitis present Cut surface mottled dull red	No increase of fibrous tissue	No increase of reticulum	1,800 gm	No increase of fibrous tissue anywhere	"

TABLE—contd

No	No of necropsy	Age	Race	Sex	Clinical note	Anatomical diagnosis at necropsy	L D B in sections	SPLEEN			LIVER.		
								Weight and gross appearance	Heidenhain's stain	Foot Bielschowsky stain	Weight and gross appearance	Heidenhain's stain	Foot-Bielschowsky stain
10	98-27	45	Hindu	Male	History of seven months' continuous fever	Enlargement of spleen, enlarged and fatty liver, chronic interstitial nephritis pyelitis, right kidney, oedema lungs, plastic pleurisy right side Necropsy 10½ hours post-mortem	+	360 gm Colour dark purplish red	No increase of fibrous tissue	No increase of reticulum	1,680 gm	No increase of fibrous tissue—marked chronic passive congestion	No increase of fibrous tissue anywhere
11	111-27	35	Hindu	Male	Kala-azar with nephritis Six months' fever Leukocytes 3,000 Hemo globin—50 per cent	Enlargement of spleen, small granular kidneys, atheroma of aorta. Necropsy twenty one hours post-mortem	+	480 gm Soft, friable Dark red in colour	No increase of fibrous tissue	Reticulum fibrils very delicate	1,230 gm Firm	Slight scattered and diffuse increase of collagen fibres, moderate chronic passive congestion	No increase of reticulum
12	117-27	27	Anglo Indian	Male	Diagnosis Kala-azar Fever eight months Leukocytes 3,000	Enlargement of spleen and liver, ulcerative colitis (amoebic) perforation of ulcer crecum, general suppurative peritonitis bilateral plastic pleurisy Necropsy 3½ hours post-mortem	+	2,400 gm Dull red in colour	No increase of fibrous tissue	Reticulum fibrils sparse and fine	2,150 gm Firm congested	Moderate patchy pericellular fibrosis not portal In portal areas, many large collections of mononuclear cells	"

13	128-27	7	Hindu	Male	Fever for eight months Diarrhoea with blood and mucus for some time before admission	Enlargement of spleen curthous liver Necropsy 2 hours post-mortem	+	510 gm Firm and some what fibrous in appearance	Trabeculae thick	Reticulum fibrils some what coarse	1,150 gm Firm	A slightly increased amount of fibrous tissue in portal areas.	"
14	141-27	38	Moham median	Male	Fever with occasional rigors and enlargement of liver and spleen of 18 months' duration Leucocytes—2,500	Enlargement of spleen Necropsy 5 hours post-mortem	+	420 gm Fibrous in appearance	Capsule thick Trabeculae coarse.	Reticulum network fairly dense and fibrils coarse in places	1,100 gm Congested in appearance	No fibrous tissue increase in portal areas	"
15	159-27	4	Hindu	Male	Fever for three months and diarrhoea for one month Diagnosis Kala azar and Bacillary dysentery	Enlargement of spleen broncho pneumonia Necropsy 6½ hours post-mortem	+++	90 gm Fairly firm Colour dark red	No increase of fibrous tissue	Fibrils fine, no definite increase	270 gm Congested	No fibrous tissue increase anywhere	"
16	160-27	35	Hindu	Male	Diagnosis Kala azar with cancer of oris	Enlargement of spleen and liver, bilateral pulmonary tuberculosis with pleurisy, hydrothorax, hydropericardium and hydro peritoneum, ulcerative colitis, plastic peritonitis Necropsy 5½ hours post mortem	+		No increase of fibrous tissue	Average appearance	1,860 gm	No fibrous tissue increase	"

TABLE—contd

No	No of necropsy	Age	Race	Sex	Clinical note	Anatomical diagnosis at necropsy	L D B in sections	SPLEEN			LIVER.		
								Weight and gross appearance	Heidenhain's stain	Foot Beels chowsky stain	Weight and gross appearance	Heidenhain's stain	Foot Beels chowsky stain
17	5-28	19	Hindu	Female	History of long continued fever and abdominal pain Had treatment for kala azar (10 injections)	Tuberculosis pulmonary, tubercles mesenterica, ulcerative enterocolitis, tuberculous fatty liver Necropsy twenty nine hours post mortem	+	210 gm Soft	No increase of fibrous tissue	No increase of reticulum fibrils	990 gm	No increase of fibrous tissue anywhere Slightly fatty	No increase of reticulum
18	6-28	40	Hindu	Male	Fever off and on for two years Diarrhoea 15 days Swelling face and neck for 15 days	Enlargement of spleen and liver, cancerous, plastic pleurisy right side, general lymphadenoid hyperplasia, ulcerative colitis, horseshoe kidney—two separate ureters Necropsy 5½ hours post-mortem	+	1,710 gm Friable surface dull dark red in colour	No increase of fibrous tissue	Reticulum fine fibrils and delicate	2,100 gm Somewhat fatty in appearance	Some portal areas show fibrosis	"
19	13-28	30	Hindu	Male	Kala azar and broncho-pneumonia Admitted moribund	Enlargement of spleen and liver, pneumonia lobar right, broncho-pneumonia left, fibrinous and plastic pleurisy general lymphadenoid hyperplasia, acute nephritis, pneumococcal meningitis fibro adenoma prostate Necropsy 13 hours post-mortem	+	1,950 gm	No thickening of capsule and trabeculae	No increase of reticulum fibrils	2,640 gm Large, firm and congested	Definite cirrhosis in places a diffuse scarring Extensive portal and pericellular fibrosis	"

20	87-28	40	Hindu	Male	Fever and shortness of breath for one month Cough Expectorations Cedema of legs Anemia	Enlargement of spleen and liver, lobar pneumonia right upper lobe, bilateral plastic pleurisy Necropsy 25 hours post-mortem	+	650 gm. Mottled dark red and purplish areas Rather soft Capsule slightly thickened	No increase of fibrous tissue, apart from the slightly thickened capsule	No definite increase of reticulum fibrils	1,380 gm Fatty in appearance	No increase of fibrous tissue Congested and markedly fatty	"
21	130-28	30	Hindu	Female	Four months fever Leukopenia and anemia Received antimony treatment	Enlargement of spleen, ovarian cyst—left Necropsy 28 hours post-mortem	+	1,170 gm Dark brownish red in colour Slight perisplenitis	No increase of fibrous tissue	Reticulum fibrils slightly coarse and more numerous than in the average case	Appears decomposed but shows no fibrosis	No increase of fibrous tissue (Fatty and congested)	"
22	154-28	26	Hindu	Female	History of the spleen being enlarged for twelve years and of fever for one year Leukopenia Probably not kala azar	Enlargement of the spleen, enlargement of the heart, general anasarca Necropsy 44 hours post-mortem	0	2,700 gm	No increase of fibrous tissue	Reticulum fibrils coarse and more numerous than in ordinary cases of Kala azar	2,130 gm	No increase of fibrous tissue (Decomposition advanced)	No increase of fibrous tissue
23	155-28	35	Hindu	Female	Fever one year Diarrhea one month Aldehyde reaction positive Received antimony	Broncho pneumonia, chronic interstitial nephritis Necropsy 32 hours post-mortem	+	210 gm Soft	Capsule thick and trabeculae fairly prominent but through out there is no increase of fibrous tissue	Reticulum fibrils sparse and fine	750 gm Soft	No increase of fibrous tissue (Fatty and congested parasites numerous)	"

TABLE—concd

No	No of necropsy	Age	Race	Sex	Clinical note	Anatomical diagnosis at necropsy	L D B in sections	SPLEEN			LIVER		
								Weight and gross appearance	Heidenhain's stain	Foot-Bielschowsky stain	Weight and gross appearance	Heidenhain's stain	Foot-Bielschowsky stain
24	156-28	20	Hindu	Female	Intermittent fever for two years	Enlargement of spleen, pyopneumothorax, right, ulcerative colitis—amoebic cirrhosis liver, jaundice Necropsy 19 hours post-mortem	+	600 gm Rather decomposed	No increase of fibrous tissue	Reticulum fibrils fine	990 gm Decomposed	Slight increase of fibrous tissue in portal areas	No increase of reticulum
25	157-28	22	Hindu	Male	Fever three months Swelling of abdomen and legs Leukopenia	Enlargement of spleen perisplenicitis, general anaemia, dilatation of heart, ankylostomiasis Necropsy 16 hours post-mortem	+	1,470 gm Colour dark red, mottled, soft	No increase of fibrous tissue	Reticulum fibrils fine	1,500 gm Fatty in appearance	No increase of fibrous tissue (Shows early passive congestion)	"
26	162-28	24	Hindu	Male	Fever six months Enlarged spleen Leukopenia	Enlargement of spleen, lobapneumonia right upper lobe, plastic pleurisy right side, congested and fatty liver Necropsy 13 hours post-mortem	+	1,290 gm Soft Colour dark red	No increase of fibrous tissue	Reticulum fibrils fine	2,130 gm Soft Appears fatty and congested	No increase of fibrous tissue (Shows passive congestion)	"
27	14-29	30	Anglo Indian	Male	Diagnosed as kala azar for four years	Enlargement of spleen and liver, adherent pericardium enlargement of mesenteric lymph nodes	0	2,891 gm Soft Colour purplish red Capsule thick	No increase of fibrous tissue	Reticulum fibrils of average appearance	1,800 gm	Slight increase of fibrous tissue in portal areas (Moderately fatty)	"

28	57-29	30	Hindu	Male	Not Kala azar	Enlarged fibrous looking spleen, pneumonia, purulent meningitis, pneumococcal, cirrhosis liver, ulcerative colitis, enlargement of mesenteric lymph nodes	0	1,540 gm Firm and fibrous in appearance	Definite fibrosis Trabeculae thick. Much collagen throughout pulp and in follicles	Reticulum fibrils increased and coarse in appearance	2,700 gm	Shows moderate grade diffuse and lobular fibrosis	No increase of reticulum
29	99-29	32	Hindu	Male	Fever two years Diarrhea six months	Enlargement of spleen, hydroperitoneum, hydrothorax, cellulitis left foot, pyonephrosis, bilateral Necropsy 13 hours post-mortem	+	820 gm Soft Dull dark red in colour	No increase of fibrous tissue	Reticulum fibrils fine	1,130 gm Firm and congested	Slight increase of fibrous tissue in some portal areas and in certain lobules, also a slight diffuse fibrosis	"
30	139-29	32	Moham medan	Male	Fever for an indefinite period Large spleen Anaemia Pyuria Edema legs Leukocytes—10,000 Aldehyde reaction +++	Enlargement of spleen, fatty, congested liver, anasarca, pyelonephritis and cystitis Necropsy 3½ hours post-mortem	+	1,650 gm Soft Typical dull dark red colour	No increase of fibrous tissue	No increase of reticulum fibrils	1,850 gm Soft Shows chronic passive congestion, no cirrhosis	No increase of collagen or reticulum in any situations	"
31	14-30	60	Hindu	Male	Cough for ten days Swelling hands and feet Malaria, (?) six months previously Liver and spleen enlarged	Plastic pleurisy right, congested and firm liver, enlarged spleen, perisplinitis, fibrosis kidney Necropsy 12 hours post mortem	+	400 gm Soft, typical dull dark red colour	No increase of fibrous tissue	No increase of reticulum fibrils	1,100 gm Firm Congested	Very slight increase of coarse collagen fibrils at angles of some portal spaces, otherwise no fibrosis.	"

DISCUSSION

In such an organ as the spleen the parasitized cells are not fixed parenchyma cells as are those of the liver. They do not seem to be extensively destroyed by the presence of the parasites within them. They are probably, however, effectively 'blocked' in their activities. One would consequently not expect in this organ fibrous tissue repair, as in other organs like the liver when essential parenchyma cells are destroyed.

In the liver, again, the cells involved directly are the similar littoral cells of Kupffer. The liver cells themselves are not immediately damaged.

From the table hepatic fibrosis is seen not to be the rule. When it occurs it may be concluded that it is the result of other direct causes, or only indirectly due to the *Leishmania* infection, which, by hampering blood regeneration, and interfering with the defences, may predispose to various intoxications.

SUMMARY

The spleens and livers from a series of 31 cases of splenomegaly, 26 of which were due to kala-azar, have been examined by special staining methods to demonstrate the fibrous tissue and reticulum.

In 80 per cent of cases of proved kala-azar the spleens showed no increase of either fibrous tissue or reticulum.

In 70 per cent of cases the livers likewise showed no increase of fibrous tissue or reticulum.

Fibrosis of the spleen and cirrhosis of the liver cannot be considered regular features of kala-azar itself.

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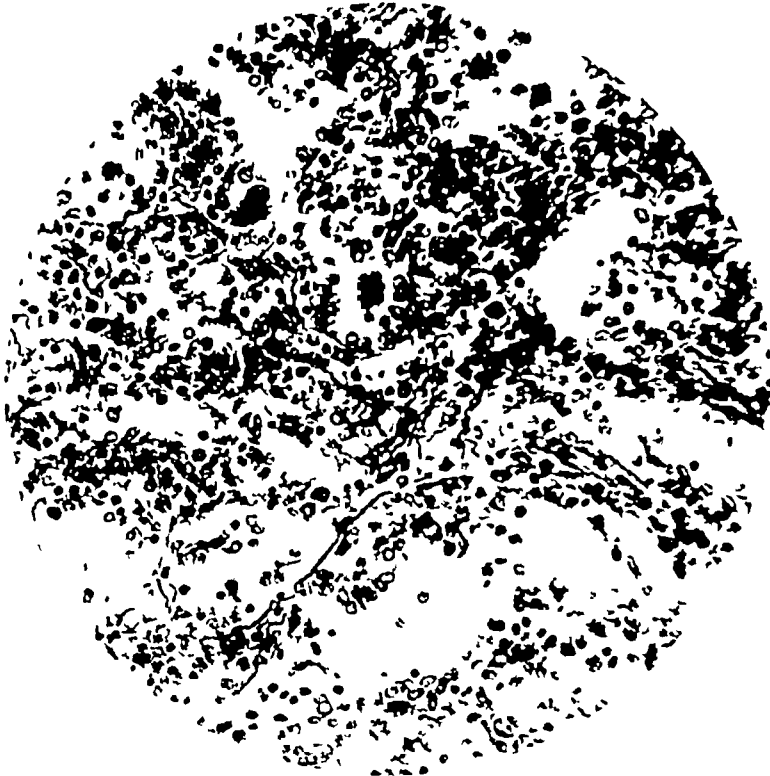


Fig 1

Spleen P M 54-27
Foot-Bielschowsky

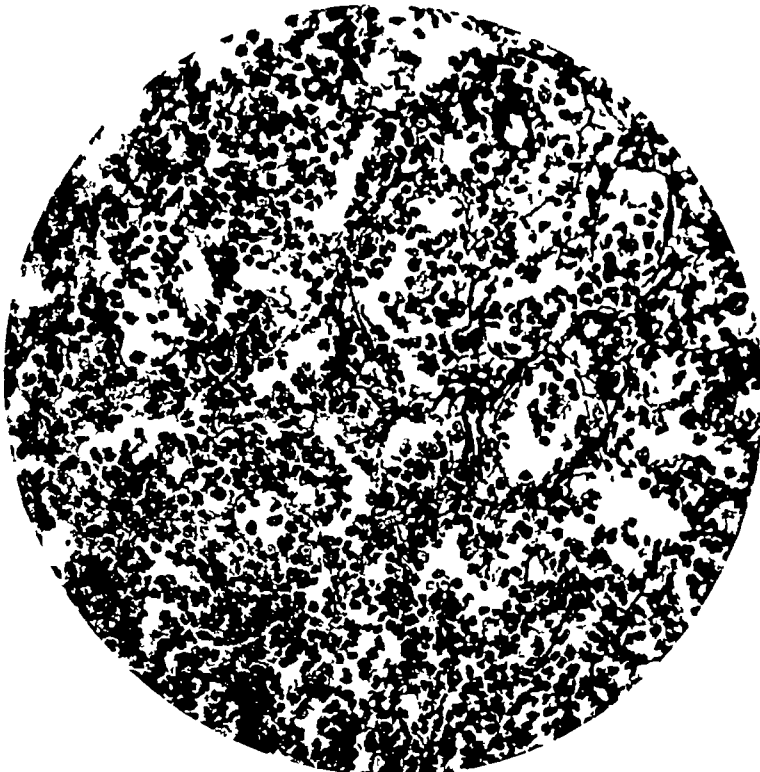


Fig 2

Spleen P M 128-27.
Foot-Bielschowsky.

PLATE XXII

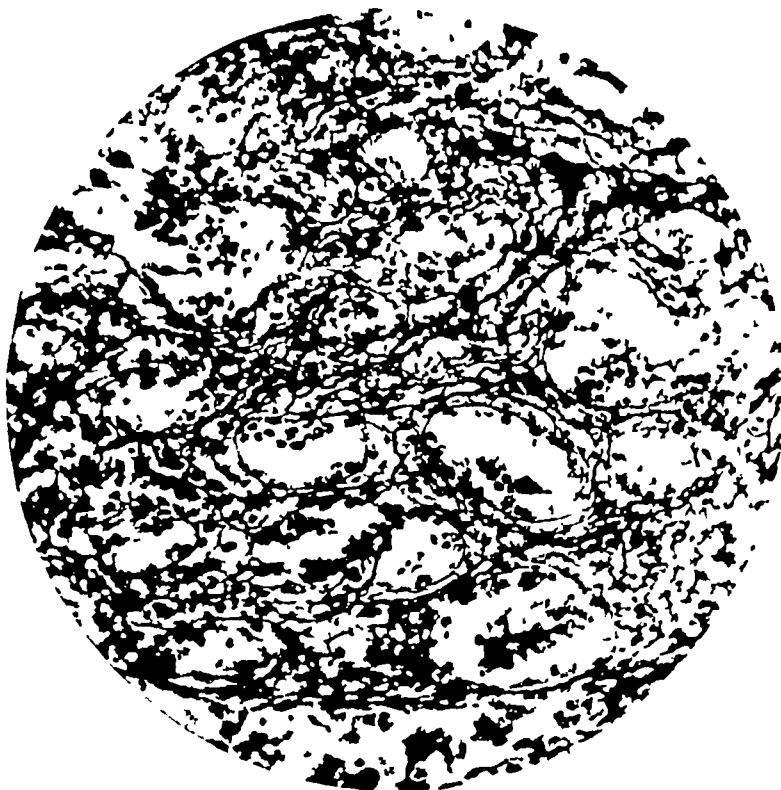


Fig 3
Spleen P M 141-27
Foot-Bielschowsky

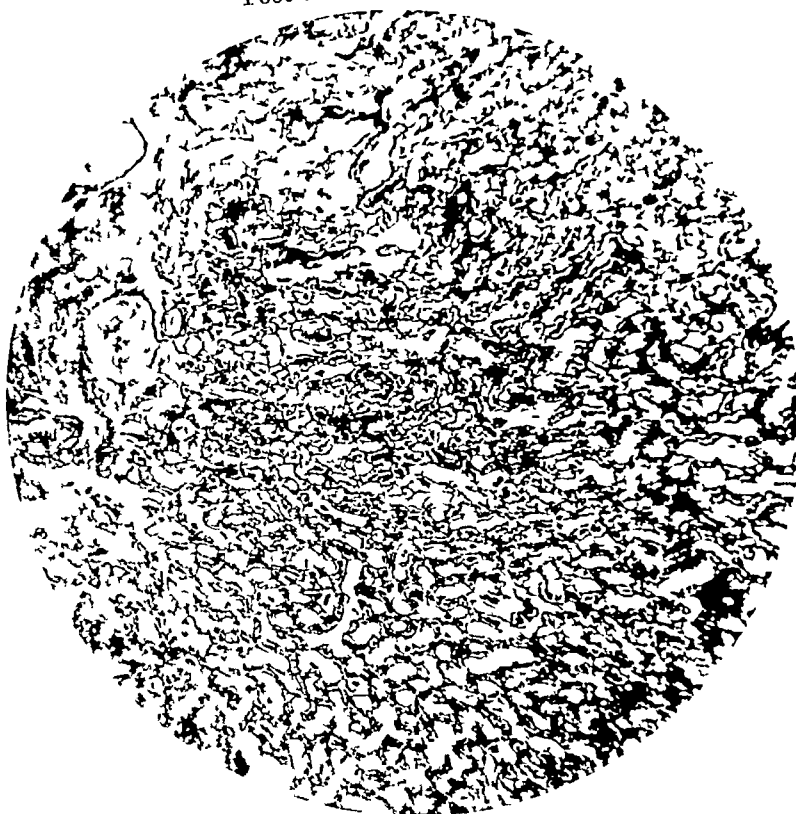
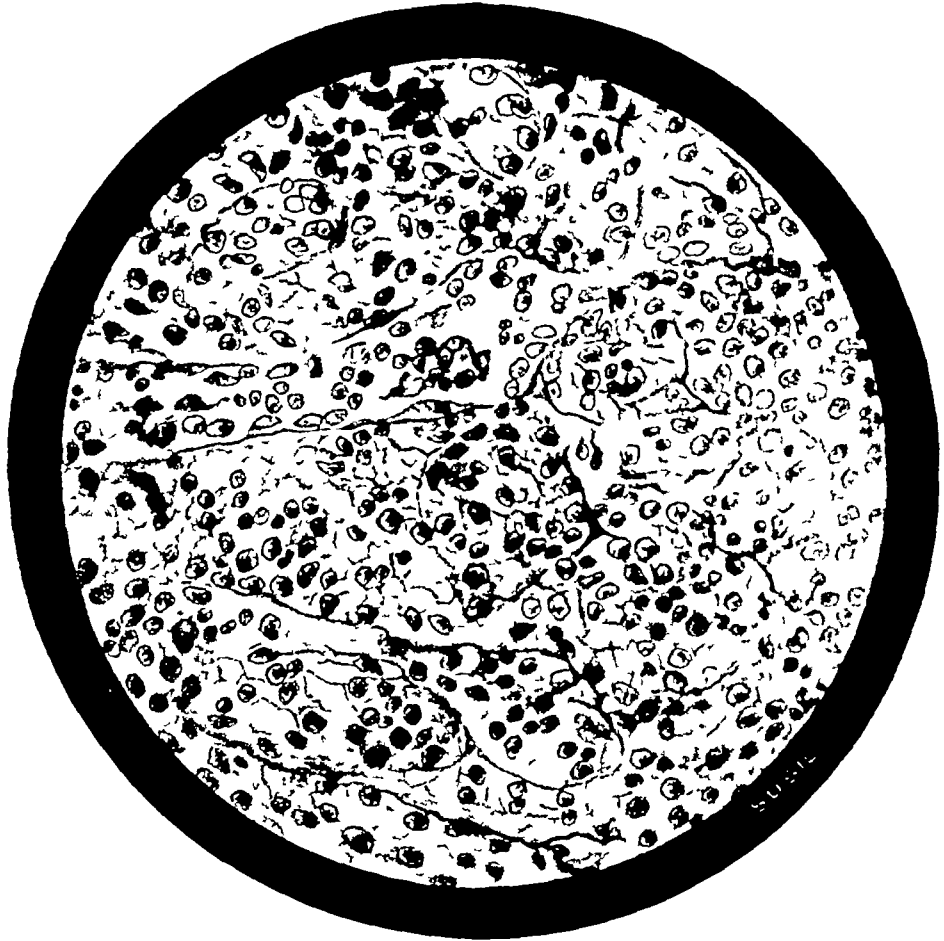


Fig 4
Liver P M 141-27
Foot-Bielschowsky

PLATE XXIII



Spleen P M 117—27
Azan Carmum Aniline Blue (Heidenham)

THE PATHOLOGY OF EPIDEMIC DROPSY.

BY

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THE disease characterized as Epidemic Dropsy is one of particular interest and importance in Bengal. The literature describing the various clinical conditions encountered in it, and discussing the ætiology and epidemiology, is voluminous. We have, however, not been able to find any detailed description of the Pathology.

A short note on some of the morbid features presented in one of our cases was written by Acton and Chopra*.

We have had the opportunity of studying the changes present in four fatal cases and the following are the results of our observations —

Case 1 —Abstract of clinical notes

The patient, a Hindu male, 40 years of age, was admitted in the service of Major E H V Hodge, I M S , on the 17th September, 1926, with dyspnoea, anasarca and cyanosis. The condition began one month prior to his admission. Clinical examination showed dilatation of the heart, arrhythmia, and oedema of the lungs. Death occurred two days later.

Necropsy 20.9.26, eleven hours after death.

Prosector Dr De

Morbid anatomy. There was general anasarca with bilateral hydrothorax, and hydropertoneum.

* *Ind Med Gazette*, LXII, 359, 1927

Skin In various situations, most markedly in the legs, there were red areas of irregular shape forming a line extending through the fatty statum subcutaneum parallel with the surface. This appearance was similar to that encountered in Case 4, and shown in Plate XXIV, fig 1

Pleural cavities Each contained about 1 000 c c of thin, pale, clear fluid

Lungs Œdematous

Pericardium A trace of fluid present. A number of small irregular red areas observed underneath the epicardium

Heart Dilated—otherwise no gross changes

Liver Passively congested

Spleen Small and congested

Stomach, Intestines, Kidneys, Pancreas, Suprarenals, Peritoneum and Omentum No gross changes

Brain Congested and œdematous

The chief gross lesions in this case were in the subcutaneous tissue

Case 2—This patient was in the service of Dr J Ferens Coltman in the Presidency General Hospital, to whom we are indebted for the clinical and post mortem notes

The patient, an Anglo Indian, 52 years of age, was admitted to hospital on 12 8 29. His illness began on 4 7 29 with headache, fever and pains in the legs. A week later a nodule appeared on the right thigh about which the skin became discoloured. Others appeared in rapid succession over the legs, abdomen, thorax, and upper extremities. About 1 8 29 he noticed swelling and œdema of the feet, ankles and legs, and a fairly severe diarrhœa.

The chief clinical features observed were œdema, and numerous nodules, described from their appearance as 'angiomatous'. They were scattered all over the body and varied in size and shape. Their average diameter was about 3 mm. There was one on the left conjunctiva.

One on the face bled freely on 14 8 29. This bleeding was controlled by pressure.

The patient died suddenly on 18 8 29.

Necropsy 18 8 29, six hours after death.

Prosector Dr J F Coltman

Morbid anatomy

Skin Dark in colour, over lower abdomen and thighs. Scattered over the entire body were numerous 'angiomatous' nodules as noted above.

Pleural cavities A small amount of slightly turbid fluid present in both. Numerous small, bright red 'petechial hæmorrhages' present over the parietal, and visceral areas of the lower lobes of both lungs.

Lungs Œdematous

Pericardium Subepicardial fat showed many small, bright red 'petechial hæmorrhages'.

Heart Enlarged and dilated, especially on the right side.

Liver Passively congested

Spleen Congested

Stomach, Intestines, Kidneys, Pancreas and Omentum No gross changes

Suprarenals Very 'mushy'

Peritoneum Under the parietal peritoneum covering the inner surface of the ribs, and diaphragm on the left side, and the liver on the right, were a number of small 'petechial hæmorrhages' and vesicles filled with clear or slightly turbid fluid.

The chief lesions in this case were in the skin, pleura, peritoneum and subepicardium.

Case 3—The patient, a Mohammedan male, 35 years of age, was admitted in the service of Dr U P Basu on the 12th August, 1929, with swelling of, and pain in, the extremities of one month's duration.

Death occurred on the 28th August.

Necropsy 29 8 29, twenty eight hours after death

Prosector Dr De

Morbid anatomy Decomposition was advanced

The anatomical diagnosis was Bilateral pleural effusion, congestion, liver, spleen and kidneys
Edema of legs

In this case the legs were œdematous and the skin below the knees presented on section an irregular, firm purplish red line through the stratum subcutaneum. The edges of this area were sharply defined. This condition was confined to the skin of the legs.

Case 4—This patient, a Hindu female, 31 years of age, was admitted in the service of Dr B Mazumdar on the 17th January, 1930, with dyspnœa palpitation, œdema of the legs and insomnia, and bleeding piles of about one month's duration. In hospital she had a low, irregular, febrile temperature, and becoming gradually worse died on 12 2 30.

Necropsy 14 2 30, forty three hours after death

Prosector Dr De

Morbid anatomy There was general anasarca with bilateral hydrothorax and hydroperitoneum.

External examination including skin. Many blotchy erythematous maculæ were present on the skin of the lower abdomen. The skin of the inguinal regions and upper areas of the thighs was thickened, firm, inelastic, rough, and relatively dark in appearance.

The skin of the back, particularly over the lumbar region, was also thick and relatively dark.

The breasts when palpated felt nodular, there being firm, lobulated, movable masses underneath.

When making the usual incisions the subcutaneous fat was seen to present a line of areas, purplish red in colour, and irregular in contour, as if lobules were infiltrated with blood.

Over the abdomen this extended to the sheath of the rectus. The dermis appeared thickened and congested (Plate XXIV, fig 1).

Over the lower abdomen the dermis was thickened and injected, and the firmness and lack of elasticity and the darkening were found to be due to the line of ' hæmorrhagic ' looking subcutaneous tissue.

Incisions were made into the skin of the thighs, legs, feet and back.

Similar appearances to those described were present (Plate XXIV, fig 2, and Plate XXV, figs 3 and 4).

The skin over the dorsum of the feet was very œdematous but less injected than that in the other situations. Features of the red areas in the subcutaneous areolar tissue, particularly over the thighs, were their firmness and sharply delimited outlines. When taken between the fingers the skin and subcutaneous tissues felt relatively hard.

On incision through the breasts the fatty connective tissue about and underneath was seen to present many areas similar to those in the subcutaneous regions. The firm palpable nodules consisted of red ' hæmorrhagic ' looking areas (Plate XXIV, fig 12). The mammary tissue itself presented its usual appearance. In the fatty tissue in the omentum, about the descending colon, and in the pelvis, these ' hæmorrhagic ' areas were almost continuous, and in it the fat was unrecognizable as such (Plate XXVI, fig 5). The appendices epiploicæ stood out very prominently as purple red nodules. The pelvic veins were enormously dilated and hæmorrhoids were present.

Further notes on internal examination —

Pleural cavity right Contains 600 c c of clear yellow fluid

Pleural cavity left Contains 1,000 c c of hæmoglobin stained fluid

Pericardial cavity Contains 120 c c of hæmoglobin stained fluid

Lungs Both are somewhat œdematous

Heart The subepicardial fat over the ventricles was much congested and appeared purplish in colour.

Liver Moderate grade of passive congestion shown

Spleen Congested in appearance

Uterus and cervix were a bright purplish red in colour

The marrow in the middle of the femur was red

The spinal cord showed marked injection of the veins in the pia (Plate XXV, fig 3, and Plate XXIV, fig 6)

MORBID HISTOLOGY

As the special features in all four cases were much the same we shall describe them together and note separately any appearances observed in any particular one of them. Descriptions of organs not presenting any characteristic changes are omitted.

Case 2 was the only one showing widespread nodular lesions of the skin but unfortunately the material was not available for microscopical study. These nodular lesions are commonly enough seen but were not present in the other three of our cases in which histological studies have been carried out.

The skin. In all cases sections show fairly marked dilatation of capillaries in the outer portion of the stratum papillare close to the epithelium (Plate XXVII, fig 7).

The inner portion of the stratum papillare and the stratum reticulare show an occasional dilated vessel (Plate XXVII, fig 8).

The fat tissue of the stratum subcutaneum shows great numbers of enormously dilated capillaries. These vessels have thin endothelial walls only (Plate XXVII, fig 9).

The dilatation of the vessels in these situations is not accompanied by any outwandering or emigration of leucocytes, and only in a few places has hæmorrhage been seen (Plate XXVIII, fig 10).

In the description of the gross appearances we several times referred to 'hæmorrhagic'-looking areas. Sections have shown that these appearances were not due to extravasations of blood, but to the extreme vascular dilatation.

The bronchi. These were examined in Cases 1, 3 and 4 and showed similar changes in each. They consist of dilatation of veins and capillaries in the submucous area, and between the glands, particularly in the former location.

The lungs. There is moderate dilatation and engorgement of the alveolar capillaries. In the alveoli are a few cells and some granular-looking material as occasionally seen in œdema of some standing.

The heart. Sections show many widely dilated vessels in the subepicardial fat (Plate XXVIII, fig 11). There are also many similarly dilated ones between the muscle fibres, particularly near the surface. In addition there are many dilated veins. The muscle fibres are sharply defined, the striations distinct and

the nuclei clear. These appearances were present in Case 1 and in Case 4, and also, but less pronounced, in Case 3.

The hypophysis. While the capillaries in the anterior portion are prominent they do not appear to vary much from the normal state.

The eye. In the ciliary processes the vessels are considerably dilated and engorged, otherwise there is little of note.

The thyroid. Little of note is observed, while some vessels are fairly full this is not a pronounced feature.

The mammae (Case 4). The breast tissue proper does not show any abnormality. The connective tissue and fat, particularly the latter, show great numbers of widely dilated and engorged vessels. Some of the lobules are crammed with them. The gross appearance is thus explained (Plate XXIV, fig. 12). There are no outwandered cells in these areas.

Mesenteric lymph nodes. These show moderate grades of capillary dilatation. In the fatty tissue about them this feature is very prominent.

The intestines generally show dilated vessels in the submucosa. Their special feature, however, consists of the enormous numbers of engorged vessels in the subperitoneal fat and in the longitudinal muscle.

The liver, spleen, kidneys, pancreas, salivary glands, sciatic and anterior tibial nerves do not present any characteristic features.

The suprarenals. In Case 1 there was no change worthv of note.

In Case 4 there was moderate vascular engorgement in the medulla with, in places, some collections of round wandering cells.

The ovary. This shows widespread dilatation of capillaries and veins (Plate XXVIII, fig. 13).

The cervix uteri. The appearance of sections of this shows from the submucosa outwards, including the muscle, very numerous, widely dilated and engorged capillaries and veins.

The body of the uterus shows similarly dilated vessels, but they are not nearly so numerous.

Striped muscles (skeletal). These in places show dilated and congested capillaries. In certain sections there are spaces between the fibres which present an 'oedematous' appearance.

The spinal cord of Case 1 was examined histologically but no noteworthy change was seen. That from Case 4 was unsuitable for sections on account of post-mortem alteration.

SUMMARY

The pathological findings in four cases of fatal epidemic dropsy are described.

In all four the chief features are similar and consist of extreme and widespread dilatation of capillaries. These dilated vessels are seen most abundantly in fatty tissue in such situations as in the stratum subcutaneum and under the pericardium and peritoneum.

In certain cases, in the skin, the dilated vessels may push the epidermis outwards, producing 'angiomatous' nodules, which may rupture and result in hæmorrhage.

The intoxication in epidemic dropsy seems to give rise to dilatation of capillaries, and this feature is most marked, apparently, in situations where the vessels have the weakest tissue support.



Fig 1

Skin from thigh Extensive vascular dilatation in subcutaneous fat



Fig 2

Skin from lumbar region—a red line of similar nature to that shown in Fig 1

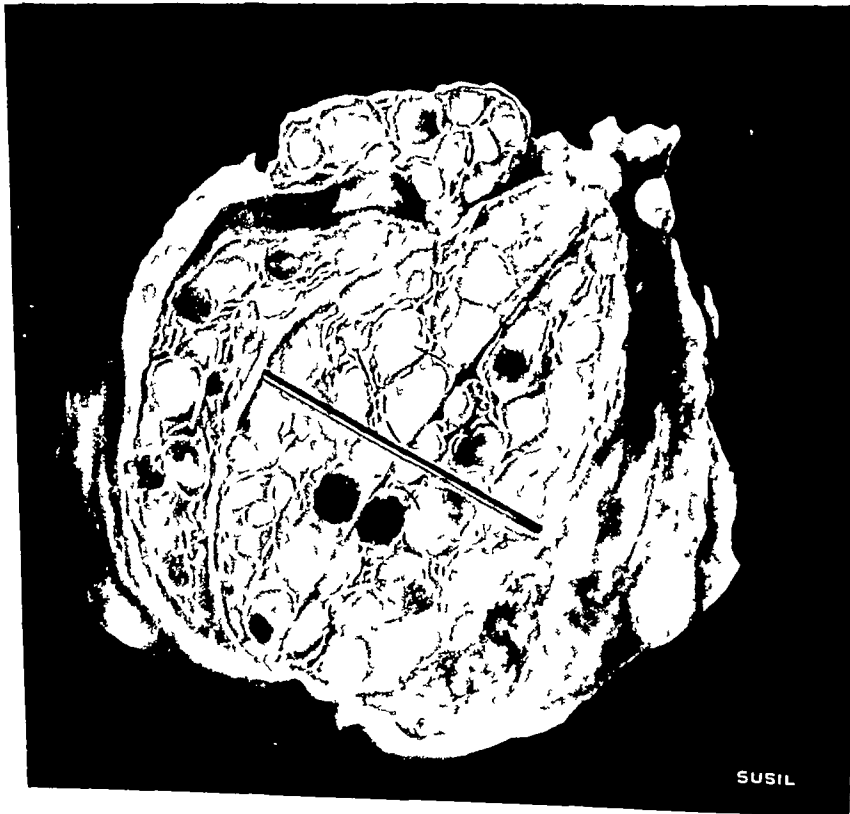


Fig 12

Breast with many fatty lobules underneath and about showing red appearance from dilatation of capillaries

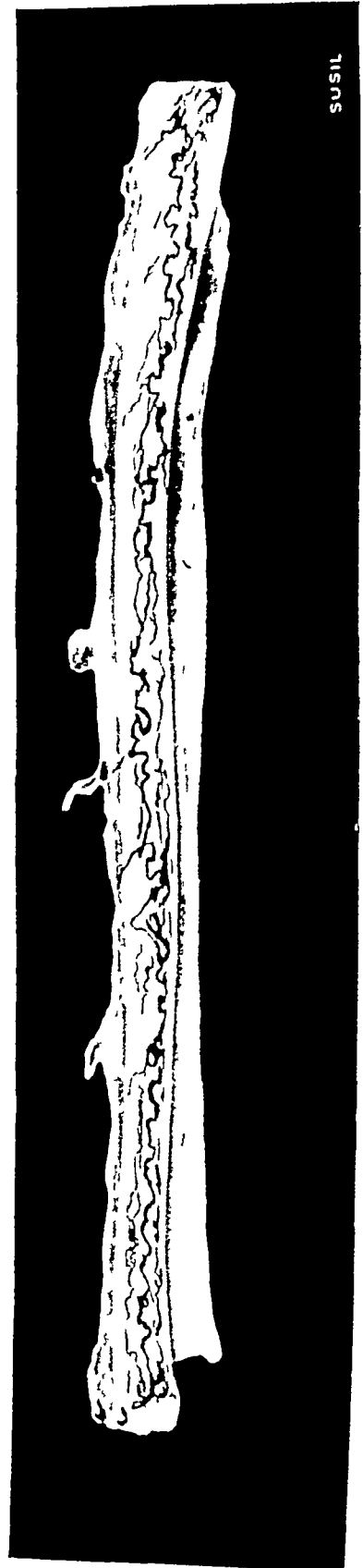


Fig. 6.
Portion of spinal cord

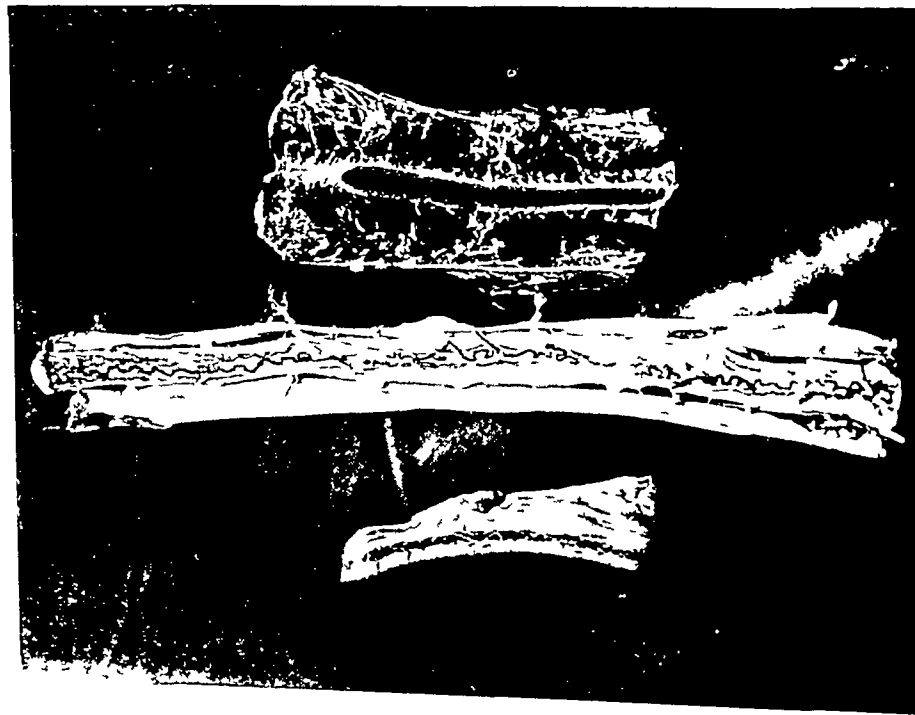


Fig 3

Photographs of two pieces of skin and of a portion of spinal cord Moderate dilatation of vessels along spinal cord

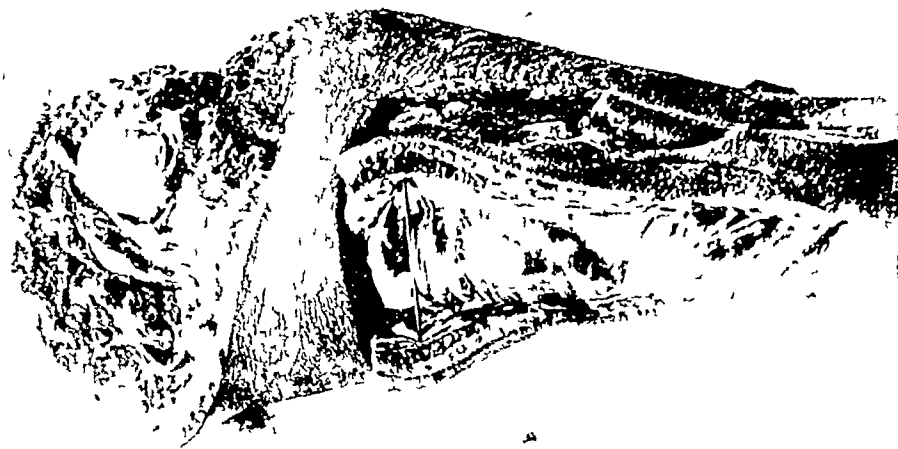


Fig 4

Photograph of thigh—the dilated vessels in stratum subcutaneum give it a black 'hemorrhagic' appearance



Fig 5

Photograph of colon showing blackish (actually when seen a deep purple) appearance in it and mesocolon, appendices epiploicae distended—all due to extremely dilated vessels



Fig 7

Skin—squamous surface—a few dilated subepithelial capillaries shown



Fig 8

Just underneath area shown in Fig 7
Dilated capillaries shown, especially
at one side

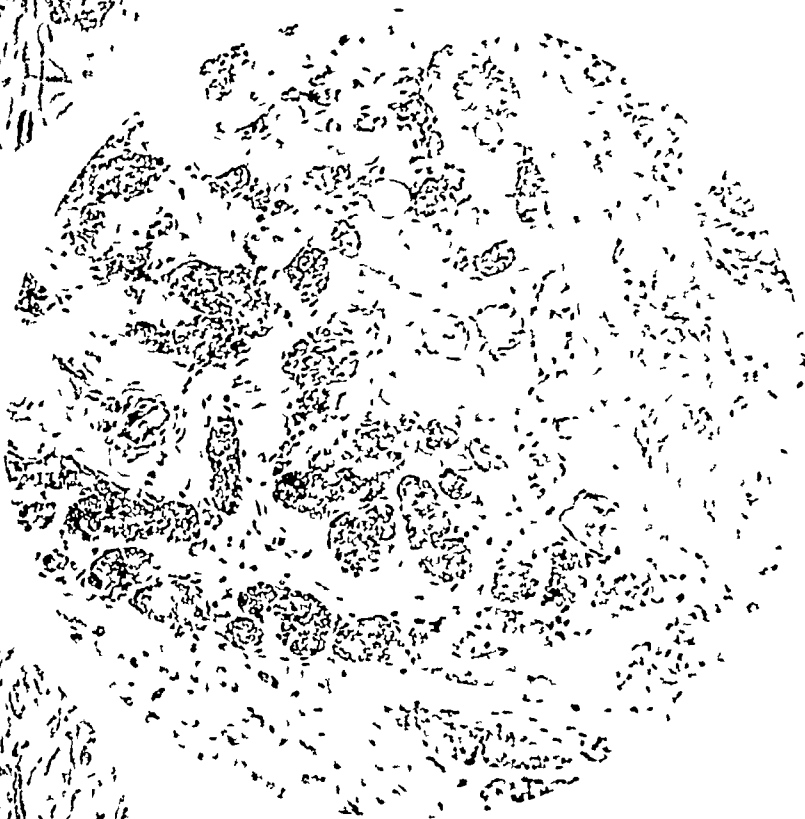


Fig 9

Photomicrograph—stratum subcutaneum—numerous greatly dilated vessels

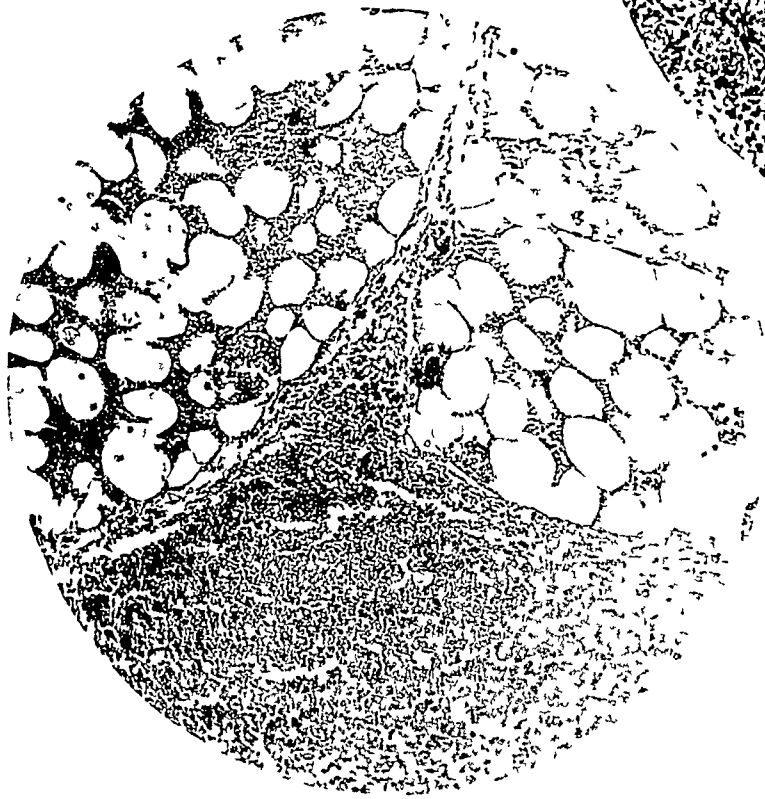


Fig 10

Fatty stratum subcutaneum with dilated vessels, plus hemorrhage



Fig 11

Pericardium and part of heart muscle
Subepicardial dilated capillaries shown

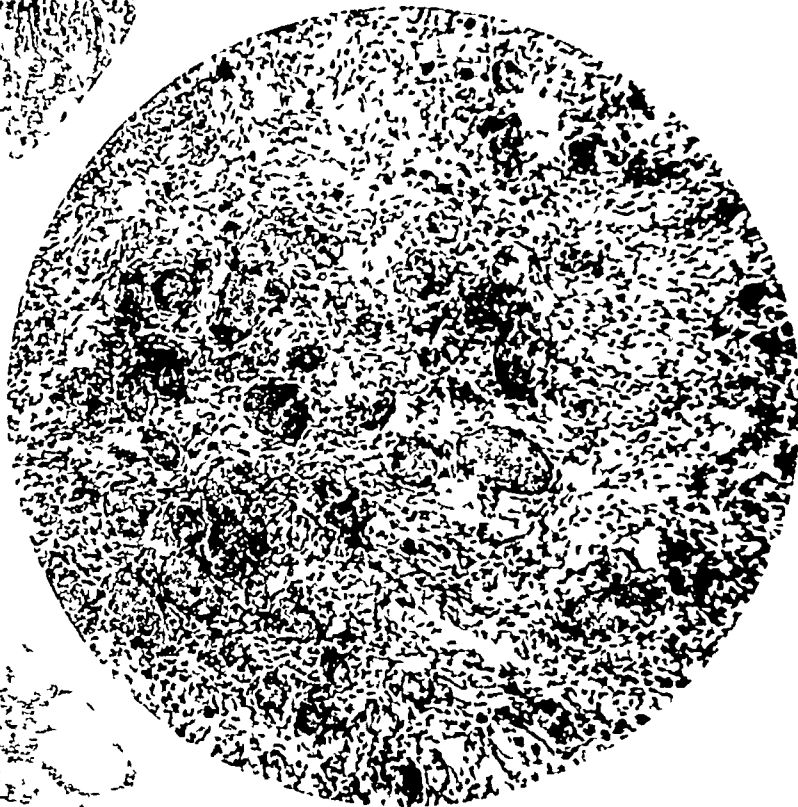


Fig 13

Dilated vessels in ovary

STUDIES IN THE NUTRITIVE VALUE OF INDIAN VEGETABLE FOOD-STUFFS.

Part II.

NUTRITIVE VALUES OF (1) BENGAL GRAM (*CICER ARIETINUM*,
LINN), (2) HORSE GRAM (*DOLICHOS BIFLORUS*),
(3) LABLAB PEA (*DOLICHOS LABLAB*)

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[Received for publication, May 1, 1931]

BENGAL gram is the most widely grown among the pulses of India and according to agricultural statistics, it accounts for nearly 13 per cent of the total area (including States) under food crops. The crop is raised all over the country, the upper basins of the Ganges and the Indus being the chief gram-producing areas. It is believed to be highly nutritious and is consumed by all classes of people and in many parts of the country it is fed to horses and cattle. The vernacular names are Sanskrit, Chenaka, Hindi, Chana, Tamil, Kadalay and Telugu, Senagalu.

Although not deemed a superior pulse, the horse gram is largely used by the poorer classes, being perhaps the cheapest of Indian pulses. In Madras and Bombay, it is an important crop and is one of the chief pulses used as cattle food. It is grown as a catch-crop and thrives with minimum amount of rainfall. As it

enriches the soil with nitrogen, the plant after harvesting the seed, is generally ploughed in as green manure. The vernacular names are Sanskrit, Kulaththa, Hindi, Kulthi, Kannada, Hurali and Tamil, Kollu.

The lablab pea is more a garden plant than an agricultural crop. It is grown all over India more or less, but is comparatively rare in Northern India and becomes abundant in Central, Western and South India. The beans are often plucked early and used as a green vegetable. When the plant turns yellow, the crop of ripe pulse is gathered and the plant is afterwards fed to cattle. The vernacular names are Pavta, Wall, Maniavare, Mochai, Avare, etc.

This paper deals with the isolation and analysis of the globulins of these seeds and also with the determination of the biological values of their total proteins.

EXPERIMENTAL

The seeds were sun-dried until crisp and easy to handle. The husks were removed from the Bengal gram and the lablab pea and the kernels ground to obtain the flour. The horse gram was ground whole as it was not possible to remove the seed-coat and also as the whole seed is invariably fed to animals. Flours passing through a 40-mesh sieve were used in the present investigation, specimens dried at 100° giving the following results on analysis —

TABLE I

Pulse	Ash	Ether extractives	Crude fibre	Crude protein	Carbohydrates (by difference)	True protein (determined separately)
<i>Cicer arietinum</i>	2.45	4.72	1.13	28.14	63.56	25.04
<i>Dolichos biflorus</i>	3.51	2.32	5.48	26.40	62.20	21.93
<i>Dolichos lablab</i>	3.45	1.73	1.15	28.74	64.93	27.40

The methods of isolation and analysis of the globulins and the determination of the biological value of the proteins have been dealt with in detail in Part I of this paper (*This Journal*, Vol XVIII, p 1217). Exactly similar methods have been employed in these experiments.

The globulins were prepared by two methods as before: (1) Dilution of the saline extract and later acidification and (2) Dialysis of the saline extract. The numbers (1) and (2) in the following tables refer to preparations by these two methods.

The globulin preparations from *Dolichos biflorus*, *Dolichos lablab* and *Cicer arietinum* were respectively light brown, white and cream-coloured. All gave reactions characteristic of proteins and contained sulphur, tyrosine and

tryptophane They were completely soluble in dilute alkali and glacial acetic acid. Elementary analysis gave the following percentages —

TABLE II

	<i>Cicer arietinum</i>		<i>Dolichos biflorus</i>		<i>Dolichos lablab</i>	
	(1)	(2)	(1)	(2)	(1)	(2)
Moisture	7.21	7.91	9.46	9.44	10.44	10.51
Ash	0.21	0.28	0.80	0.74	0.58	0.54
On ash and moisture free basis						
Nitrogen	16.91	16.87	15.38	15.76	15.59	15.59
Sulphur	0.54	0.56	0.33	0.37	0.44	0.16

The results of the Van Slyke analyses of these preparations and of individual determinations of the amino acids in them are given in Tables III and IV. Cystine was estimated by the more recent method of Remington (1930).

TABLE III

Analysis of the globulin preparations by the Van Slyke method
(Expressed as per cent of total nitrogen)

Form of nitrogen	Globulin of <i>Dolichos biflorus</i>		Globulin of <i>Cicer arietinum</i>		Globulin of <i>Dolichos lablab</i>	
	(1)	(2)	(1)	(2)	(1)	(2)
Acid insoluble melanin	1.32	1.03	0.66	0.49	0.98	0.86
Acid soluble melanin (absorbed by lime)	0.13	0.12	0.58	0.32	0.42	0.41
Amide	11.19	11.34	10.47	10.58	9.09	8.09
Diamino —						
Arginine	12.12	12.69	20.48	19.95	16.99	16.82
Histidine	6.07	4.35	2.25	2.33	2.12	2.66
Cystine	0.70	0.59	0.92	0.96	0.69	0.73
Lysine	9.73	10.54	8.35	9.02	8.76	9.22
Mono amino —						
Amino	59.71	59.32	56.82	56.12	56.82	56.75
Non amino	1.30	1.93	0.71	0.82	3.96	3.58
TOTAL	102.27	101.91	101.24	100.59	99.83	100.02

TABLE III—concl'd

Form of nitrogen	Globulin of <i>Dolichos biflorus</i>		Globulin of <i>Cicer arietinum</i>		Globulin of <i>Dolichos lablab</i>	
Free amino N in the native proteins						
	(1)	(2)	(1)	(2)	(1)	(2)
By direct estimation	4.92	4.95	4.74	4.67	4.47	4.55
Half lysine nitrogen	4.87	5.27	4.18	4.51	4.38	4.61

TABLE IV

Expressed as per cent of protein (ash and moisture-free)

Amino acid	Globulin of <i>Dolichos biflorus</i>	Globulin of <i>Cicer arietinum</i>	Globulin of <i>Dolichos lablab</i>	Method
Lysine	8.15	7.42	7.27	Van Slyke
Histidine	2.38	1.42	1.38	"
Arginine	6.00	10.29	8.09	"
"	7.07	11.85	8.72	Plimmer and Rosedale (1925)
Cystine	1.24	2.02	1.54	Remington (1930)
Tyrosine	4.01	2.95	4.34	Fohn and Marenzie (1929)
"	5.74	4.90	5.11	Zuwerkalao (1926)
Tryptophane	0.76	0.46	0.44	Fohn and Marenzie (1929)
"	1.02	0.46	0.42	Tillmans and Alt (1925)

Feeding experiments were carried out at a 10 per cent level of protein intake instead of at 5 per cent which was previously found to be lower than the minimum

required for maintenance For preparing the rations the whole pulse flour was used and not the isolated globulins and therefore the biological values obtained refer to the total proteins in the seed The percentage composition of the rations is given below —

TABLE V

	Non protein ration	Protein ration
Cane sugar	10	10
Butter fat	8	8
Cod liver oil	2	2
Agar agar	1	0
Salt mixture	4	2.5
Flour		Enough to contain 10 per cent pro tein (N \times 6.25)
Starch	75	To make up to 100

The flour was mixed with sufficient water and cooked on a boiling water bath for 30 minutes The starch, sugar and the salts were then added and the cooking continued until the starch was completely gelatinized After cooling, the fats were mixed in thoroughly and the mixture spread out in thin layers on glass plates and dried at a temperature of about 60°—70° and finally powdered

Apart from its food, each rat was fed daily with 3—6 drops of cod-liver oil and 50 mg of dried brewer's yeast containing 2.9 mg nitrogen which, however, has not been taken into account in calculating the metabolism data given in Table VI In addition to the biological values, the digestibilities of the proteins have also been calculated from the equation

$$\text{Percentage digestibility} = \frac{\text{Absorbed N} \times 100}{\text{Total intake of N}}$$

In Table VII are given the net protein values of the three pulses calculated as follows (Mitchell, 1923) —

$$\text{Net protein value} = \frac{\text{Content of digestible protein} \times \text{biological value}}{100}$$

TABLE VI
Metabolism data (daily averages)

Rat No	Initial weight	Final weight	Food intake	Nitrogen intake	Faecal nitrogen	Urinary nitrogen	Metabolic nitrogen in faeces per gramme of food	Endogenous nitrogen in urine per 100 g body weight	Food nitrogen in faeces	Absorbed nitrogen	Food nitrogen in urine	Total nitrogen retained	Biological value	Digestibility
	g	g	g	mg	mg	mg	mg	mg	mg	mg	mg	mg	per cent	per cent
<div>Period I Protein free ration N=0.084 per cent</div>														
13	90.0	84.5	4.47	3.76	12.84	19.21	2.87	22.01						
14	80.5	74.5	4.20	3.54	10.21	18.55	2.43	23.93						
15	92.5	88.0	5.19	4.37	13.45	20.17	2.59	22.97						
16	77.0	70.0	3.31	2.79	10.32	17.82	3.11	24.25						
17	69.5	66.1	4.06	3.42	8.52	15.85	2.09	23.38						
18	81.9	75.6	4.25	3.57	11.07	18.32	2.60	23.26						
<div>Period II <i>Dolichos biflorus</i> flour ration N=1.75 per cent</div>														
13	84.1	82.5	4.31	75.43	42.74	31.70		29.38	46.05	14.28	31.77	69	61	
14	73.5	70.8	3.90	69.84	38.90	31.70		28.33	41.51	14.83	26.08	64	59	
15	83.0	81.5	4.70	82.24	52.86	33.86		30.64	51.60	16.00	35.60	69	63	
16	68.5	69.0	3.72	65.09	38.18	27.19		26.39	38.70	11.79	26.91	70	59	
17	63.0	63.0	3.65	63.87	34.74	26.65		26.53	37.34	13.07	24.27	65	58	
18	74.4	74.6	4.06	71.04	41.18	30.25		30.35	40.69	11.08	26.61	65	57	
												Average	67	79

J, MR	Period III <i>Cicer arietinum</i> flour ration N=1 705 per cent										7								
	13	14	15	16	17	18	6 30	87 5	83 0	107 40		48 14	33 86	27 16	80 24	16 96	63 28	79	75
							5 63 <td>85 0<td>79 3<td>95 96<td>39 24<td>33 67<td>22 41<td>73 55<td>16 47<td>60 54<td>82</td><td>77</td></td></td></td></td></td></td></td></td></td>	85 0 <td>79 3<td>95 96<td>39 24<td>33 67<td>22 41<td>73 55<td>16 47<td>60 54<td>82</td><td>77</td></td></td></td></td></td></td></td></td>	79 3 <td>95 96<td>39 24<td>33 67<td>22 41<td>73 55<td>16 47<td>60 54<td>82</td><td>77</td></td></td></td></td></td></td></td>	95 96 <td>39 24<td>33 67<td>22 41<td>73 55<td>16 47<td>60 54<td>82</td><td>77</td></td></td></td></td></td></td>	39 24 <td>33 67<td>22 41<td>73 55<td>16 47<td>60 54<td>82</td><td>77</td></td></td></td></td></td>	33 67 <td>22 41<td>73 55<td>16 47<td>60 54<td>82</td><td>77</td></td></td></td></td>	22 41 <td>73 55<td>16 47<td>60 54<td>82</td><td>77</td></td></td></td>	73 55 <td>16 47<td>60 54<td>82</td><td>77</td></td></td>	16 47 <td>60 54<td>82</td><td>77</td></td>	60 54 <td>82</td> <td>77</td>	82	77	
							7 70 <td>92 0<td>84 5<td>131 20<td>49 98<td>41 30<td>29 88<td>101 32<td>23 25<td>77 07<td>76</td><td>77</td></td></td></td></td></td></td></td></td></td>	92 0 <td>84 5<td>131 20<td>49 98<td>41 30<td>29 88<td>101 32<td>23 25<td>77 07<td>76</td><td>77</td></td></td></td></td></td></td></td></td>	84 5 <td>131 20<td>49 98<td>41 30<td>29 88<td>101 32<td>23 25<td>77 07<td>76</td><td>77</td></td></td></td></td></td></td></td>	131 20 <td>49 98<td>41 30<td>29 88<td>101 32<td>23 25<td>77 07<td>76</td><td>77</td></td></td></td></td></td></td>	49 98 <td>41 30<td>29 88<td>101 32<td>23 25<td>77 07<td>76</td><td>77</td></td></td></td></td></td>	41 30 <td>29 88<td>101 32<td>23 25<td>77 07<td>76</td><td>77</td></td></td></td></td>	29 88 <td>101 32<td>23 25<td>77 07<td>76</td><td>77</td></td></td></td>	101 32 <td>23 25<td>77 07<td>76</td><td>77</td></td></td>	23 25 <td>77 07<td>76</td><td>77</td></td>	77 07 <td>76</td> <td>77</td>	76	77	
							5 62 <td>77 5<td>73 0<td>95 79<td>40 02<td>31 70<td>21 87<td>73 92<td>16 24<td>57 08<td>77</td><td>77</td></td></td></td></td></td></td></td></td></td>	77 5 <td>73 0<td>95 79<td>40 02<td>31 70<td>21 87<td>73 92<td>16 24<td>57 08<td>77</td><td>77</td></td></td></td></td></td></td></td></td>	73 0 <td>95 79<td>40 02<td>31 70<td>21 87<td>73 92<td>16 24<td>57 08<td>77</td><td>77</td></td></td></td></td></td></td></td>	95 79 <td>40 02<td>31 70<td>21 87<td>73 92<td>16 24<td>57 08<td>77</td><td>77</td></td></td></td></td></td></td>	40 02 <td>31 70<td>21 87<td>73 92<td>16 24<td>57 08<td>77</td><td>77</td></td></td></td></td></td>	31 70 <td>21 87<td>73 92<td>16 24<td>57 08<td>77</td><td>77</td></td></td></td></td>	21 87 <td>73 92<td>16 24<td>57 08<td>77</td><td>77</td></td></td></td>	73 92 <td>16 24<td>57 08<td>77</td><td>77</td></td></td>	16 24 <td>57 08<td>77</td><td>77</td></td>	57 08 <td>77</td> <td>77</td>	77	77	
							6 25 <td>72 1<td>66 0<td>106 50<td>43 22<td>30 53<td>28 16<td>78 34<td>16 89<td>61 45<td>78</td><td>74</td></td></td></td></td></td></td></td></td></td>	72 1 <td>66 0<td>106 50<td>43 22<td>30 53<td>28 16<td>78 34<td>16 89<td>61 45<td>78</td><td>74</td></td></td></td></td></td></td></td></td>	66 0 <td>106 50<td>43 22<td>30 53<td>28 16<td>78 34<td>16 89<td>61 45<td>78</td><td>74</td></td></td></td></td></td></td></td>	106 50 <td>43 22<td>30 53<td>28 16<td>78 34<td>16 89<td>61 45<td>78</td><td>74</td></td></td></td></td></td></td>	43 22 <td>30 53<td>28 16<td>78 34<td>16 89<td>61 45<td>78</td><td>74</td></td></td></td></td></td>	30 53 <td>28 16<td>78 34<td>16 89<td>61 45<td>78</td><td>74</td></td></td></td></td>	28 16 <td>78 34<td>16 89<td>61 45<td>78</td><td>74</td></td></td></td>	78 34 <td>16 89<td>61 45<td>78</td><td>74</td></td></td>	16 89 <td>61 45<td>78</td><td>74</td></td>	61 45 <td>78</td> <td>74</td>	78	74	
							6 30 <td>82 8<td>77 2<td>107 40<td>44 12<td>34 21<td>25 98<td>81 42<td>18 10<td>63 32<td>78</td><td>76</td></td></td></td></td></td></td></td></td></td>	82 8 <td>77 2<td>107 40<td>44 12<td>34 21<td>25 98<td>81 42<td>18 10<td>63 32<td>78</td><td>76</td></td></td></td></td></td></td></td></td>	77 2 <td>107 40<td>44 12<td>34 21<td>25 98<td>81 42<td>18 10<td>63 32<td>78</td><td>76</td></td></td></td></td></td></td></td>	107 40 <td>44 12<td>34 21<td>25 98<td>81 42<td>18 10<td>63 32<td>78</td><td>76</td></td></td></td></td></td></td>	44 12 <td>34 21<td>25 98<td>81 42<td>18 10<td>63 32<td>78</td><td>76</td></td></td></td></td></td>	34 21 <td>25 98<td>81 42<td>18 10<td>63 32<td>78</td><td>76</td></td></td></td></td>	25 98 <td>81 42<td>18 10<td>63 32<td>78</td><td>76</td></td></td></td>	81 42 <td>18 10<td>63 32<td>78</td><td>76</td></td></td>	18 10 <td>63 32<td>78</td><td>76</td></td>	63 32 <td>78</td> <td>76</td>	78	76	
															Average		78	76	
	Period IV <i>Dolichos lablab</i> flour ration N=1 80 per cent										88*								
	13	14	15	16	17	18	5 57	87 1	86 5	100 20		54 69	27 19	34 76	65 44	10 95	57 58	54	65
							4 20 <td>81 0<td>82 0<td>75 60<td>40 62<td>38 19<td>26 89<td>48 71<td>22 35<td>26 36<th>54</th><th>64</th></td></td></td></td></td></td></td></td></td>	81 0 <td>82 0<td>75 60<td>40 62<td>38 19<td>26 89<td>48 71<td>22 35<td>26 36<th>54</th><th>64</th></td></td></td></td></td></td></td></td>	82 0 <td>75 60<td>40 62<td>38 19<td>26 89<td>48 71<td>22 35<td>26 36<th>54</th><th>64</th></td></td></td></td></td></td></td>	75 60 <td>40 62<td>38 19<td>26 89<td>48 71<td>22 35<td>26 36<th>54</th><th>64</th></td></td></td></td></td></td>	40 62 <td>38 19<td>26 89<td>48 71<td>22 35<td>26 36<th>54</th><th>64</th></td></td></td></td></td>	38 19 <td>26 89<td>48 71<td>22 35<td>26 36<th>54</th><th>64</th></td></td></td></td>	26 89 <td>48 71<td>22 35<td>26 36<th>54</th><th>64</th></td></td></td>	48 71 <td>22 35<td>26 36<th>54</th><th>64</th></td></td>	22 35 <td>26 36<th>54</th><th>64</th></td>	26 36 <th>54</th> <th>64</th>	54	64	
							5 50 <td>86 0<td>87 1<td>99 02<td>48 87<td>40 22<td>34 46<td>64 56<td>26 60<td>37 96<th>59</th><th>65</th></td></td></td></td></td></td></td></td></td>	86 0 <td>87 1<td>99 02<td>48 87<td>40 22<td>34 46<td>64 56<td>26 60<td>37 96<th>59</th><th>65</th></td></td></td></td></td></td></td></td>	87 1 <td>99 02<td>48 87<td>40 22<td>34 46<td>64 56<td>26 60<td>37 96<th>59</th><th>65</th></td></td></td></td></td></td></td>	99 02 <td>48 87<td>40 22<td>34 46<td>64 56<td>26 60<td>37 96<th>59</th><th>65</th></td></td></td></td></td></td>	48 87 <td>40 22<td>34 46<td>64 56<td>26 60<td>37 96<th>59</th><th>65</th></td></td></td></td></td>	40 22 <td>34 46<td>64 56<td>26 60<td>37 96<th>59</th><th>65</th></td></td></td></td>	34 46 <td>64 56<td>26 60<td>37 96<th>59</th><th>65</th></td></td></td>	64 56 <td>26 60<td>37 96<th>59</th><th>65</th></td></td>	26 60 <td>37 96<th>59</th><th>65</th></td>	37 96 <th>59</th> <th>65</th>	59	65	
							4 70 <td>73 8<td>75 1<td>84 61<td>47 66<td>37 74<td>32 20<td>52 41<td>23 84<td>28 57<th>55</th><th>62</th></td></td></td></td></td></td></td></td></td>	73 8 <td>75 1<td>84 61<td>47 66<td>37 74<td>32 20<td>52 41<td>23 84<td>28 57<th>55</th><th>62</th></td></td></td></td></td></td></td></td>	75 1 <td>84 61<td>47 66<td>37 74<td>32 20<td>52 41<td>23 84<td>28 57<th>55</th><th>62</th></td></td></td></td></td></td></td>	84 61 <td>47 66<td>37 74<td>32 20<td>52 41<td>23 84<td>28 57<th>55</th><th>62</th></td></td></td></td></td></td>	47 66 <td>37 74<td>32 20<td>52 41<td>23 84<td>28 57<th>55</th><th>62</th></td></td></td></td></td>	37 74 <td>32 20<td>52 41<td>23 84<td>28 57<th>55</th><th>62</th></td></td></td></td>	32 20 <td>52 41<td>23 84<td>28 57<th>55</th><th>62</th></td></td></td>	52 41 <td>23 84<td>28 57<th>55</th><th>62</th></td></td>	23 84 <td>28 57<th>55</th><th>62</th></td>	28 57 <th>55</th> <th>62</th>	55	62	
							4 33 <td>68 0<td>69 0<td>77 94<td>37 51<td>33 81<td>26 38<td>51 56<td>21 54<td>30 02<th>58</th><th>66</th></td></td></td></td></td></td></td></td></td>	68 0 <td>69 0<td>77 94<td>37 51<td>33 81<td>26 38<td>51 56<td>21 54<td>30 02<th>58</th><th>66</th></td></td></td></td></td></td></td></td>	69 0 <td>77 94<td>37 51<td>33 81<td>26 38<td>51 56<td>21 54<td>30 02<th>58</th><th>66</th></td></td></td></td></td></td></td>	77 94 <td>37 51<td>33 81<td>26 38<td>51 56<td>21 54<td>30 02<th>58</th><th>66</th></td></td></td></td></td></td>	37 51 <td>33 81<td>26 38<td>51 56<td>21 54<td>30 02<th>58</th><th>66</th></td></td></td></td></td>	33 81 <td>26 38<td>51 56<td>21 54<td>30 02<th>58</th><th>66</th></td></td></td></td>	26 38 <td>51 56<td>21 54<td>30 02<th>58</th><th>66</th></td></td></td>	51 56 <td>21 54<td>30 02<th>58</th><th>66</th></td></td>	21 54 <td>30 02<th>58</th><th>66</th></td>	30 02 <th>58</th> <th>66</th>	58	66	
							4 86 <td>79 2<td>79 9<td>87 48<td>45 87<td>38 03<td>31 19<td>56 29<td>21 24<td>33 05<th>59</th><th>64</th></td></td></td></td></td></td></td></td></td>	79 2 <td>79 9<td>87 48<td>45 87<td>38 03<td>31 19<td>56 29<td>21 24<td>33 05<th>59</th><th>64</th></td></td></td></td></td></td></td></td>	79 9 <td>87 48<td>45 87<td>38 03<td>31 19<td>56 29<td>21 24<td>33 05<th>59</th><th>64</th></td></td></td></td></td></td></td>	87 48 <td>45 87<td>38 03<td>31 19<td>56 29<td>21 24<td>33 05<th>59</th><th>64</th></td></td></td></td></td></td>	45 87 <td>38 03<td>31 19<td>56 29<td>21 24<td>33 05<th>59</th><th>64</th></td></td></td></td></td>	38 03 <td>31 19<td>56 29<td>21 24<td>33 05<th>59</th><th>64</th></td></td></td></td>	31 19 <td>56 29<td>21 24<td>33 05<th>59</th><th>64</th></td></td></td>	56 29 <td>21 24<td>33 05<th>59</th><th>64</th></td></td>	21 24 <td>33 05<th>59</th><th>64</th></td>	33 05 <th>59</th> <th>64</th>	59	64	
															Average		57	65	

Period V Protein-free ration N=084 per cent

	13	14	15	16	17	18	284	749	833	1280	1394	380	1762
							337	725	782	1278	1351	353	1792
							460	800	840	1211	1405	263	1793
							373	675	724	1246	1178	334	1683
							370	620	675	1003	1044	271	1611
							380	734	771	1203	1274	317	1701

* This figure has not been taken into account in calculating the average biological value

TABLE VII

Pulse	Total protein (N \times 6.25) Per cent	Net protein value Per cent
<i>Dolichos biflorus</i>	26.40	10.43
<i>Cicer arietinum</i>	28.14	16.68
<i>Dolichos lablab</i>	28.74	10.65

The analysis of the three globulins (Tables II, III and IV) show that the different preparations of each of the globulins are identical in composition. The globulin of *Cicer arietinum* contains more arginine than is usually met with in globulins. The globulin of *Dolichos biflorus* contains a higher percentage of tryptophane than the other two.

From the metabolism data, the average biological values at a 10 per cent level of intake of the total proteins of the pulses *Dolichos biflorus*, *Cicer arietinum* and *Dolichos lablab* are respectively 67, 78 and 57, while their percentage digestibilities at the same level of intake are 59, 76 and 65 respectively. The total protein of *Cicer arietinum* is therefore not only more digestible, but also better assimilated than the proteins of the other two pulses. Hence its net protein value (Table VII) also is higher. The protein of *Dolichos lablab* is more digestible, but has a lower biological value than that of *Dolichos biflorus*.

SUMMARY

The globulins of *Cicer arietinum*, *Dolichos biflorus* and *Dolichos lablab* were isolated and analysed by the method of Van Slyke. Tyrosine, tryptophane, cystine and arginine were estimated by separate methods. A determination of the biological values of these three pulses has been carried out by means of feeding experiments on rats at a ten per cent level of protein intake. Among them, *Cicer arietinum* appears to be the best protein food because of its high net protein value.

The expenses of this investigation were met by a grant from the Indian Research Fund Association.

Our best thanks are due to Dr Jivraj N. Mehta, M.D., M.R.C.P., Dean, Seth G. S. Medical College, for the encouragement he has given us so freely and to Col. R. McCarrison, C.I.E., M.D., D.Sc., F.R.C.P., I.M.S., Director, Nutritional Research, I.R.F.A., Pasteur Institute, Coonoor, for suggesting the use of the

Coonoor type of cage for the metabolism experiments. A slightly modified form of the above cage is now being used with satisfactory results.

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FURTHER OBSERVATIONS ON THE DIETS AND MATERNITY CONDITIONS OF WOMEN WORKERS IN BOMBAY.

BY

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[Received for publication, May 6, 1931]

DURING previous years the members of the Maternal Mortality Inquiry have investigated the social conditions of women of the industrial class in the city with special reference to their diet (Wills and Talpade, 1930), the conditions during pregnancy and the result to the children born (Balfour and Talpade, 1930). The author has continued these studies during the past year with a view to confirming and extending the earlier observations. As formerly the inquiry was limited to women mill-workers and to the wives of mill-workers. The methods of study were the same as previously reported (Wills and Talpade, 1930).

For the present investigation 45 women were selected for detailed study. These cases were drawn largely from the Hindu working class and included wives of workers. Roughly half were Ghatees, people from the Ghats, and half Konkanees, people from the coast. This selection was made as social workers (personal communication) have stated that the diets of these two classes were very different. When at their native place this may be so, because of the varying nature of the local food supply but in Bombay circumstances compelled them to buy in the cheapest market with the result that their diets were very similar. These two classes were further subdivided into workers and wives of workers. For the benefit of European readers it must be stated that all the women were married and the majority had had children.

Tables I and II show the result obtained in the present inquiry and for purposes of comparison some of those previously reported. The results are very similar to those previously recorded. As before the diet is remarkably low in animal products, especially animal fat and the total fat content is extremely low. The grains eaten

however include whole grains such as *bagree* (pearl millet) and *patni* (unmilled rice) which are good sources of vitamin B and salts, and contain more protein than the milled grains. These facts may explain the immunity from anæmia of pregnancy of these working class women. In other respects, however, the diet is very poor as not only is the supply of essential food-stuffs low but the quality of the supplies, especially the vegetables, milk and fish is very inferior.

A detailed analysis of the diets of the different subdivisions studied is given in Table II. There is no significant difference between the diets of the Ghatees and the Konkanees, though the latter have a slightly more liberal diet. The diet of the workers is, however, higher than that of the wives and furthermore the diet of the present series of cases is definitely more liberal than that of the series previously studied. These facts are remarkable and led to an examination of other data available to see if there is any explanation of these changes. It is at once seen that the women workers are in a far better position than the wives of mill-workers, for if from the united wages of the family the rent was deducted and then the money available per head for food and clothing is calculated on the same basis as the diet (Wills and Talpade, 1930) (Lusk's coefficients factor), it is found that the women workers have Rs 17-14-0 per month whereas the wives had only Rs 9-4-0. This fact explains the more liberal diet of the workers. The question then arises as to why the mill class as a whole are better off than a year ago and therefore able to afford a more liberal diet. The explanation is probably that when the previous survey was made many of the mills were working short-time, so that the monthly wage was reduced and further that the price of all food has fallen very considerably this year.

Now though the workers are better off and better fed than the wives, it is a remarkable fact that the average weight of the babies of the non-workers (5 lb 15 oz this series) is greater than that of the workers (5 lb 11 oz). The average weight of both has, however, risen as compared with the previous findings (workers 5 lb 8 oz, wives 5 lb 12 oz) (Balfour and Talpade, 1930). This is of considerable importance as demonstrating the importance of the food supply in this connection. As pointed out in the earlier paper (Balfour and Talpade, 1930), the diet of Indian industrial workers is so deficient that it reacts on the foetus and leads to the birth of infants that are significantly lower in weight than the average infants of the same race and place but belonging to the non-industrial classes. In England this is not so, for the disabilities of the industrial class only affect the child after birth: at birth the infant is of average weight and size. This year in Bombay the average weight of the infants from this small series of industrial workers was nearly approximate to the mean for the non-industrial classes, presumably owing to the more liberal diet. It is to be regretted that another control series was not obtained, as it is possible that this too would show a higher average birth weight. The fact

TABLE I

Average daily diet in ounces of women mill-workers and mill-workers' wives

No	Classes		
1	Women mill-workers ²¹		
		Milk	0 70
		Ghee	0 07
		Vegetable oil	0 88
		Meat	1 20
		Chicken	2 00
		Fish	0 80
		Sugar	0 57
		Polished rice	7 70
		Patni	3 70
		Jwari	0 16
		Bayree	1 17
		Atta	0 19
		White bread	0 17
		Dal and gram	1 15
		Potato	0 66
		Onion	0 98
		Green	0 39
		Bimjal	0 51
		Pulpy vegetable	0 39
		Fruit	0 09
		Coco nut	0 30
		Gogary.	0 01
2	Mill workers' wives ²²		
		Milk	0 56
		Ghee	0 02
		Vegetable oil	0 91
		Meat	0 18
		Chicken	0 91
		Sugar	0 63
		Polished rice	6 92
		Patni	1 90
		Jwari	0 81
		Bayree	3 31
		Atta	0 19
		White bread	0 08
		Dal and gram	1 16
		Potato	0 61
		Onion	0 53
		Green	0 61
		Bimjal	0 16
		Pulpy vegetable	0 19
		Fruit	0 00
		Coco nut	0 47
		Gogary.	0 04

TABLE II

Daily average food consumption, blood count, housing and income of mill-workers

No	Classes	DAILY INTAKE PER PERSON IN GRAMS						Vitamins Average daily intake in arbitrary units			Red blood cells per c mm in millions	White blood cells per c mm	Hæmoglobin per cent, 100 per cent= 13.8 grs	Income per woman for food and clothes in rupees
		Total protein	Animal protein	Total fat	Animal fat	Carbohydrates	Calories	A	B	C				
1	Old series	M 50	8.0	26.0	2.0	40.4	2,121	21.0	92	30	3.75	3,769		7.29
	20	d 16	4.0	3.0	2.0	15.7	655	10.0	39	12	0.392	602		
2	Present series— All classes	M 57	14.0	38.0	3.0	41.3	2,234	21.0	98	39	4.006	8,420	72.42	M 13.41
	45	d 17	7.5	15.0	1.8	12.0	592	15.0	53	19	0.501	2,561	8.53	d 3.14
3	Kokanees	M 60	13.0	42.5	2.0	43.1	2,334							
	25	d 18	7.1	17.0	2.0	7.9	533							
4	Ghatees	M 54	14.0	33.0	3.0	39.2	2,078							
	18	d 19	8.2	10.5	2.0	13.8	643							
5	Wives	M 52.5	13.5	37.0	3.0	37.5	2,074	18.00	77	39				M 9.4
	24	d 15	6.0	15.0	1.5	10.1	536	13.00	39	16				d 3.5
6	Workers	M 62	13.5	38.0	4.0	45.8	2,428	25.60	120	40				M 17.14
	21	d 18	9.0	16.0	2.0	12.2	570	15.00	59	21				d 6.06

M=Mean

d=Standard deviation

that the average weight of the workers' babies also rose is, no doubt as stated above, related to the greater food intake though the weight still falls short of that of the wives' babies, owing presumably to the injurious effect of work during the latter months. Another factor that may operate in improving the birth weight is the maternity benefit which is now available in all the mills.

In conclusion I wish to thank the Medical Officers of the Wadia Hospital for permission to investigate their cases and to Dr. Lucy Wills for help with this work.

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STILL-BIRTH AND NEO-NATAL DEATH IN INDIA A PRELIMINARY INQUIRY *

BY

CHRISTINE J THOMSON, M D, Ph D (St A),
Lady Irwin Research Worker, India, 1929-30.

[Received for publication, May 7, 1931]

THE following is a brief summary of the main results obtained during a survey of the cases of still-birth and neo-natal death occurring in a large number of Indian hospitals during the year 1929—full data regarding this investigation being given in a detailed report now in the press

The work was undertaken under the auspices of the Lady Irwin Research Fund (as part of a larger inquiry into Maternal and Infantile Mortality being carried on by Dr M I Balfour and her colleagues under the Indian Research Fund Association), and was commenced at the Haffkine Institute, Bombay, and completed in Madras. At the end of the inquiry, Delhi and Calcutta were also visited by the writer, and a large number of hospital data personally collected.

The investigation comprised a series of 200 post-mortem examinations of still-born fetuses, or cases of neo-natal death, together with a classification of over 3,700 similar cases obtained from hospital registers by means of an All-India Questionnaire. Owing to various modifying factors in India such as the prevalence of tropical diseases; vitamin deficiencies, social customs (such as *purdah*) which render the expectant mother more liable to disease, difficulty regarding adequate care during confinement, the problem of obtaining sufficient post-mortem examinations, etc—the results obtained are of considerable interest, when compared with the findings of observers in the West.

* The full report of which this paper is a summary, is obtainable from —

(a) The Secretary, Countess of Dufferin's Fund, Viceregal Estates, Simla. Price Re 1
Or from

(b) Messrs H K Lewis & Sons, Booksellers, Gower Street, London, W C 1. Price 1s 6d
(491)

I POST-MORTEM SERIES

The following were the primary causes of death in the 200 post-mortem cases —

Primary cause of death	Percentage
Complications of labour	19 5
Ante-partum hæmorrhage	13 5
Toxæmias of pregnancy	15 5
Syphilis (a) 'certain'	12 5
(b) 'possible'	4 0
Acute maternal disease (tropical, etc)	13 4
Fœtal states	8 0
Placental disease	5 5
Prematurity <i>per se</i>	3 5
Neo-natal states	2 0
Cause unknown—(macerated)	3 0
	<hr/>
	100 0

Seventy-five per cent of these cases were premature. The community-distribution among the 194 mothers (6 being mothers of twins) was as follows —

Hindus	151
Mohammedans	15
Christians (both Anglo-Indian and Indian)	28
	<hr/>
	194

(1) COMPLICATIONS OF LABOUR

(a) *Birth-traumata among the total cases* — Exclusive of 85 macerated fœtuses, 21 cases among the 200 showed tentorial tears, with or without tears of the falx cerebri, while a further 3 had tears of the latter alone.

Among breech deliveries, 41.4 per cent showed septal tears, while the percentage for vertex presentations was only 15.3. One-third of the total cases of septal tears occurred, however, in spontaneous vertex deliveries. Cerebral hæmorrhage was of course present in the great majority of these cases, while gross visceral hæmorrhage was found in 35 instances.

(b) *Cases dying primarily from the complications of labour* — The figure of 19.5 per cent for this group was obviously an under-estimate, as, owing to the impossibility of obtaining consecutive post-mortems, the series contained an undue proportion of macerated fœtuses.

The complications of labour included contracted pelvis, malpresentations, prolapse of the cord, and dystocia from various causes, including forceps delivery.

The preponderance of multiparæ in cases of malpresentation was noteworthy, while a history of any ante-natal care was generally absent

(2) ANTE-PARTUM HÆMORRHAGE

Sixteen foetal or neo-natal deaths were due to accidental hæmorrhage, and 11 to placenta prævia, or a total of 13·5 per cent. Cases of retro-placental hæmatoma have been included under 'placental disease'

Ante-partum hæmorrhage, when it occurs in India, takes the usual heavy toll of foetal and neo-natal life, but does not account for such a large percentage of the total still-births, etc., as in the West

The association of accidental hæmorrhage with malaria awaits further investigation

(3) TOXÆMIAS OF PREGNANCY

India presents features of great interest, with regard to the toxæmias of pregnancy, and especially with reference to the striking geographical distribution of eclampsia

In the series of 200 cases, 15·5 per cent of the deaths were due to maternal toxæmia, while the writer's personally-collected data showed that in Calcutta, during 1929, there were 10 times as many still-births and neo-natal deaths from eclampsia as in Bombay, and more than half again as many as in Madras. The maternal mortality among cases of eclampsia was 32·14 per cent in Calcutta, as compared with 9·42 per cent in Madras

Among hospital deliveries, there was a much higher incidence of the disease among Mohammedans—a finding in accordance with the observations of other workers

(4) SYPHILIS

The data obtained during the present inquiry, both with regard to serological tests on various series of maternity cases, and with reference to the post-mortem examinations, suggest that at least in certain cities of India, this disease, as a primary cause of still-birth and neo-natal death, has been popularly over-estimated

(a) With regard to Bombay, out of 372 consecutive labour cases, 13·6 per cent gave positive blood-reactions

(b) With regard to Madras, out of 1,000 similar cases, 12·5 per cent were positive

It will be recalled that a similar enquiry by J. N. Cruikshank in Glasgow (1924) showed that the incidence of positive blood-tests was between 9 per cent and 10 per cent in patients of the hospital class

In the present series of 200 cases, the incidence of 'certain' syphilis was 14·5 per cent but including 'probable' and 'possible' cases, the figure was

18.5 per cent. As a primary cause of death, the percentage due to the disease was 16.5. Of the instances of 'certain' syphilis, practically 90 per cent were spirochæte-positive.

Among the cases of syphilis, 83.7 per cent were macerated, while 81 per cent of the total were premature.

Although the great majority of syphilitic cases are usually macerated, the converse does not hold good, for the present series, for example, only slightly more than 1 case, out of every 3 macerated, was syphilitic.

(5) TROPICAL DISEASES

In the present series, only 10.5 per cent of the still-births or neo-natal deaths could be ascribed to tropical diseases *per se*. It must be remembered, however, that the inquiry was limited, for all practical purposes, to urban populations, and therefore did not include malarial areas such as the Punjab.

(A) The incidence of malaria was unavoidably under-estimated in the inquiry, since only cases which definitely showed parasites in the blood could legitimately be included. Instances classified as 'fever of unknown origin' must in some cases at least have been in reality malaria.

In a Bombay series of 83 placental smears, 3.6 per cent were positive for parasites.

(B) Although 'anæmia of pregnancy' is one of the most formidable causes of maternal mortality in India, this disease only accounted for 2 per cent of the foetal or neo-natal deaths in the series. This figure, for various reasons discussed in the Report, is also probably an under-estimate, as the writer found, for example, that in 1928, the percentage was 7.5 for similar cases occurring at the largest maternity hospital in Calcutta.

(C) No foetal death occurred from osteomalacia in the present series, but it must be remembered that the examinations were mainly carried out in Southern India where this disease is practically unknown.

Numbers of cases were of course reported via the All-India Questionnaire.

(D) No deaths were primarily due to ankylostomiasis.

(E) Dysentery and 'acute enteritis' accounted for 2.5 per cent while

(F) 2.5 per cent were classified as due to 'fever of unknown origin'. No cases were due to kala-azar, beri-beri, nor other tropical disease.

(G) Apart from specifically tropical affections, 2.5 per cent of the still-births or neo-natal deaths were due to acute or chronic maternal diseases, such as pneumonia or valvular disease of the heart.

(6) FETAL STATES

These conditions (which include monstrosities, hydramnios, visceral hæmorrhages of apparently spontaneous origin, etc.) accounted for 8 per cent of the deaths.

in the post-mortem series Syphilis appeared to play no part in their causation

A feature of considerable interest in the present inquiry was the number of cases of malformation or disease of the urinary tract. Apart from instances of gross associated congenital abnormalities (e.g., anencephalus), there were no fewer than 8 examples of this type of lesion, 2 of these having already been classified under deaths from 'foetal states'

(7) DISEASES OF THE PLACENTA

Eleven deaths in the series, or 5.5 per cent, were primarily due to 'placental states'

Out of a total of 144 placentas examined, no fewer than 57 showed evidence of gross disease or abnormality

An interesting finding in connection with all the placentas examined was the presence of leucocytosis in no fewer than 10.4 per cent of the specimens. This subject is worthy of further investigation

(8) PREMATUREITY AND NEO-NATAL STATES

A large amount of research is still required, with regard to the question of prematurity in India and the following notes are necessarily limited in their scope —

(A) With regard to the proportion of children prematurely born in any ordinary series of viable births the writer found that out of a series of 1,077 consecutive deliveries in Calcutta, 18.2 per cent were premature the proportions varying according to community from 9.8 per cent for Europeans and Anglo-Indians, to 39.1 per cent for Mohammedans (who, however, formed a very small proportion of the total births)

The figures for Hindus and Indian Christians were intermediate being 23.6 and 20.4 per cent respectively

(B) With regard to prematurity *versus* maturity in 1,561 cases of still-birth and neo-natal death occurring in Bombay, Madras and Calcutta, the percentages of such foetuses prematurely born were 64.5, 64.4, and 59.2 respectively

(C) With reference to the proportion of foetal or neo-natal deaths *primarily due to prematurity* in the actual post-mortem series, the figure was 3.5 per cent. The Questionnaire figures for all-India (which, of course, were not based upon post-mortems) showed, however, a much higher incidence than this

With regard to 'neo-natal states' (which include 'pneumonia neonatorum,' pulmonary hæmorrhages, etc.), only 2 per cent of cases in the present series were primarily due to these causes

Five cases of 'pneumonia neonatorum' were found in the series, including a case classified under 'neo-natal states'

It must be remembered that post-mortems are unusually difficult to obtain, in instances of neo-natal death, and that these findings are based upon a small number of cases

(9) FŒTAL MACERATION

Three per cent of the cases in the post-mortem series had to be classified under 'maceration—cause unknown'

II ALL-INDIA QUESTIONNAIRE

This Questionnaire, regarding cases of still-birth and early neo-natal death occurring among hospital in-patients for the year 1929, was sent out to all the women's hospitals administered by the Countess of Dufferin's Fund through the kindness of Dr A C Scott, C M O, W M S I, while additional copies were forwarded by the writer to a number of Government and Mission hospitals throughout the country

The returns deal with 3,715 cases, which had not, of course, been subjected to post-mortem examinations. The data obtained were consequently much more reliable for conditions such as the complications of labour, ante-partum hæmorrhage, eclampsia, and foetal monstrosities, than for syphilis, prematurity *per se*, and neo-natal death, etc

The figures for the primary causes of death were found to differ very widely for the various districts of India, but the main findings may be summarized, briefly, as follows —

(1) In 24 out of the 29 tabulated districts, the percentage of foetal or neo-natal deaths primarily due to the complications of labour appeared to be higher than in Great Britain, while in the remaining 5 districts (including Bombay, Calcutta and Madras), the figures closely approximated to the British findings

With regard to contracted pelvis, which was mainly due to osteomalacia, the large number of multiparæ compared with primiparæ, was in striking contrast to the usual findings in the West

The proportion of foetuses suffering craniotomy in cases of contracted pelvis, was almost identical with the percentage found in Great Britain

Among very premature foetuses, a large number were malpresentations which might have spontaneously rectified themselves, had gestation been prolonged to full-term

(2) Ante-partum hæmorrhage showed in general a much lower incidence than Great Britain

(3) The same statement holds good for the toxæmias of pregnancy, with several exceptions—notably Calcutta. The figure for Delhi and Agra were exceptionally low

(4) The very low figures for syphilis were clearly an under-statement, since many of the macerated foetuses classified under 'cause unknown' must obviously have been due to this disease

(5) The incidence of 'acute maternal disease' (mainly tropical affections) as a cause of still-birth and neo-natal death was found, of course, to be much higher in India than in the West—the figures varying from 11 per cent to 18 per cent for the great cities, as compared with an average British figure of 2.6 per cent. The highest percentages were found in Karachi and Shikarpur, although the series in both cities was small. Many neo-natal deaths occurred in Bombay as a result of maternal malaria.

(6) 'Foetal states' do not appear to be more prevalent in India than in the West.

(7) With regard to prematurity *per se*, the Questionnaire figures appear to show, even in the absence of post-mortem examinations, that a considerably higher percentage die from marked prematurity in India than in Great Britain. The average British figure is 3.2 per cent while the largest Indian cities varied from 12 per cent to 17 per cent excluding the many malpresentations in reality due to prematurity.

(8) Data relative to 'neo-natal states' were obviously of doubtful value, in the absence of pathological examinations.

Of the total neo-natal deaths tabulated as from all causes, 87.22 per cent occurred in the first week of life, while 37.88 per cent took place in the first 24 hours of existence.

(9) Practically no cases in the Questionnaire returns were classified as due to placental disease.

(10) The percentage of macerated foetuses dying from 'causes unknown' varied greatly in the different districts.

(11) With reference to community, in the majority of places the still-birth rate among Mohammedans was in general significantly higher than among Hindus, while, with the exception of Lahore, the figure for 'Christians and others' was the lowest of the three communities.

References to the findings of other workers, together with full appendices dealing with both the post-mortem and All-India Questionnaire cases, will be found in the Report* which also contains suggestions regarding preventive measures relative to still-birth and neo-natal death in India.

Thanks have already been expressed in the main Report, to the many who rendered assistance during the various stages of the inquiry, but the writer desires, in connection with the Countess of Dufferin's Fund, again to thank Dr A. C. Scott,

* Obtainable from the Secretary, Countess of Dufferin's Fund, Viceregal Estates, Simla
Price Re 1
J, MR

Chief Medical Officer, W M S I , and the Superintendents and Staffs of the Dufferin Hospitals throughout India, for help so freely given during the present investigation

She also wishes to place on record her appreciation of the services of her Indian women-assistants services, Dr H Patil (now of the W M S I), Bombay , and Doctors M Chacko, Kanthamani, and C Nair, Government Hospital for Women and Children, Madras

THE DISTRIBUTION OF *ANOPHELES LUDLOWII* IN BENGAL AND ITS IMPORTANCE IN MALARIAL EPIDEMIOLOGY.*

BY

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[Received for publication, May 9, 1931]

Anopheles ludlowi Theobald is a Far Eastern species with a distribution ranging from India to Malaya, Siam, the Dutch East Indies, Borneo and the Philippine Islands. In India it has a very limited range of distribution and it occurs only in the Andaman Islands on the coastal tract of Lower Burma and in Lower Bengal. Previous records of *A. ludlowi* from Bengal are from one district only, namely, 24-Perganas and from three localities in that district (Fraserganj, Konkondigee and Port Canning). Even in these three places this anopheline was none too common. Because of its very limited distribution and sparse prevalence, malarialogists in Bengal naturally ignored this species as being of no importance in the epidemiology of malaria in the province.

* Read before the Indian Science Congress held at Nagpur in January 1931.

† The distribution of *Anopheles ludlowi* in India mentioned in this paper is based on records which are accepted generally and which have been subsequently confirmed by later workers. There are, however, some reports of *A. ludlowi* from a few other localities which are omitted here as they are not generally accepted and these are the records from (1) Goa, (2) Daman in Guzerat, (3) Colombo and (4) Madras City. Covell (1927, *Ind Med Res Memoir*, No 5, p 15) considers that the records of *A. ludlowi* from Goa and Daman are not entirely certain. In fact, this species has not been observed anywhere on the west coast of India. The record of *A. ludlowi* from Colombo has not been confirmed by subsequent workers and Carter (1925) thinks that the presence of this species in Ceylon is doubtful. The record of this species from Madras City is a mention by Hodgson (1911 *Ind Jour Med Res*, I, 4) of Horne's finding of the species in that city. This record is a very doubtful one. None of the subsequent workers, including the present author, ever found *A. ludlowi* in Madras. Some very intensive investigations were carried out by Dr K R Rao as Special Malaria Officer to the City of Madras and he never came across this species there, nor for that matter anywhere on the Madras coast, even though, as he informs the author in a letter, he made a particular search for this species. In a recent paper (Rao, 1930, *Records of the Malaria Survey of India*, I 1, Dec), he makes no mention at all of *A. ludlowi* in his list of anophelines found in the Madras Presidency. The Madras record is therefore a very doubtful one. From the undoubted records now available, it seems likely that Bengal is the western limit of distribution of *Anopheles ludlowi*.

The author's investigations show that *Anopheles ludlowii* has a much wider distribution in Bengal than is commonly supposed and that in many areas it occurs in very large numbers. Although this species is commonly considered as a sea-coast species, it has been found in the interior of Lower Bengal on the banks of tidal rivers, and in some instances in places far removed from the sea-coast. Moreover, *Anopheles ludlowii* has been observed to be highly susceptible to infection with malaria parasites and to have an abnormally high natural infection rate. It has also been found to be responsible for an epidemic of malaria of a severe type in the vicinity of Budge-Budge in the district of 24-Perganas and in Chengail in Howrah district. In view of the occurrence of *Anopheles ludlowii* in several parts of Lower Bengal and its high susceptibility to infection even under natural conditions, it is essential that it should be recognized as an important transmitter of malarial infection in Bengal.

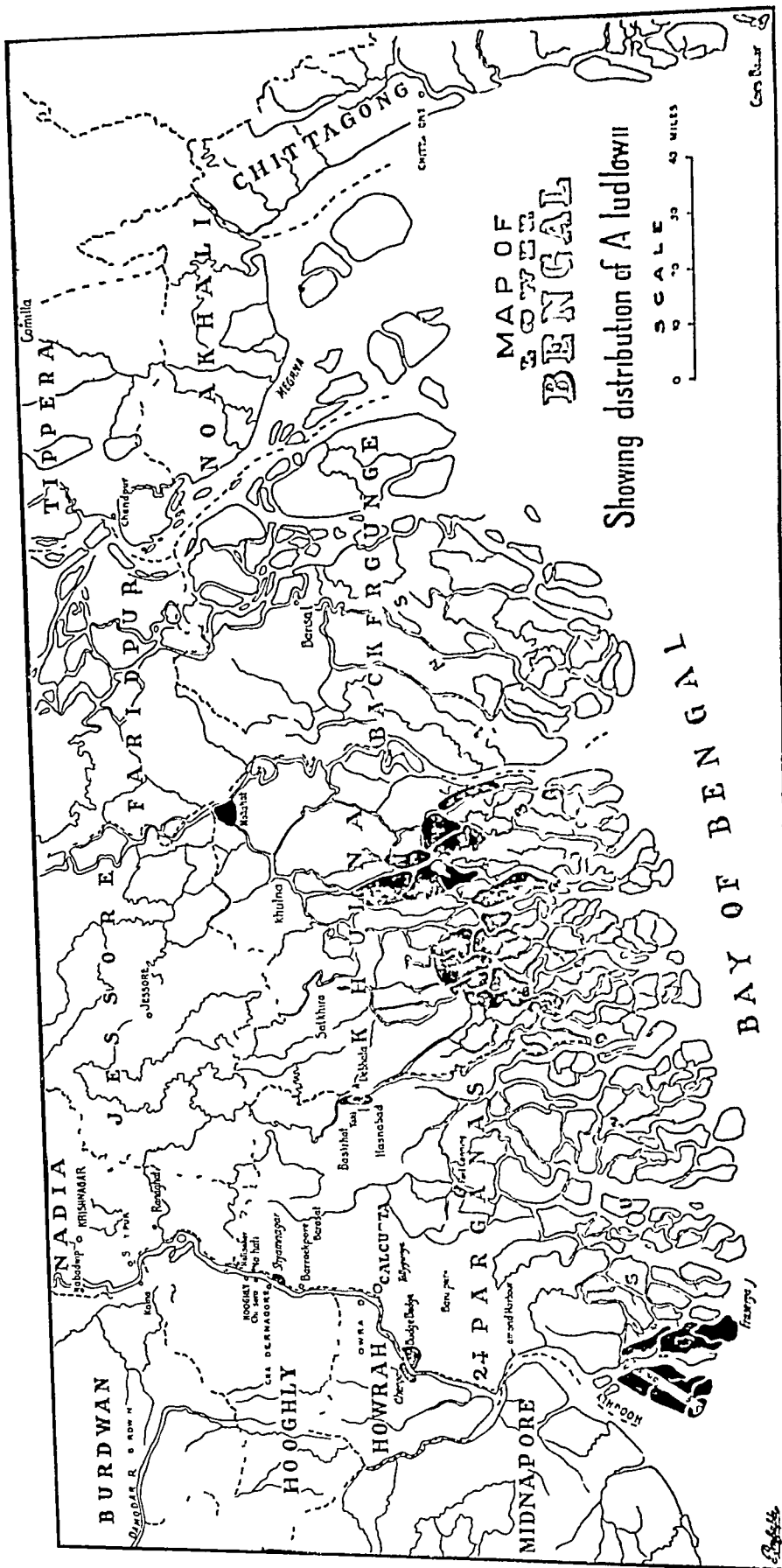
***Anopheles ludlowii* distribution in Bengal**

Anopheles ludlowii is a brackish-water breeder occurring usually in areas close to the sea-board. In Bengal, we find that while it occurs in areas close to the sea, it also extends very far inland, even as much as a hundred miles inland from the sea-coast. It should be observed, however, that it occurs mostly on the banks of tidal channels.

From the information now available as the result of surveys and investigations in Bengal, *Anopheles ludlowii* has been observed to occur in three districts in Lower Bengal, namely, Howrah, 24-Perganas and Khulna,* the localities where it occurs in these districts are marked on the accompanying map (see Map 1). In the district of Howrah, *A. ludlowii* occurs in the vicinity of Chengail on the right bank of the river Hooghly [Chengail, Rajbansipara, Premchand Mill, Ludlow Mill, Sisberia, Bauria, Paschim Bauria, Chak Kasi]. In the district of 24-Perganas it occurs in Saugor Islands on the sea-board [Fuldobi, Beguakhali], at Port Canning on the river Matla, at Budge-Budge on the left bank of the Hooghly [Jaychandipur, Abhirampur, Kalipur, Chorail, Radhakantapur, Orient Mill, Lothian Mill, Albion Mill, Badli Kalmagar], at Shamnagar which is situated 19 miles to the north of Calcutta on the left bank of the Hooghly, and at Hasnabad on the right bank of the Ichamati [Chengrighata, Sayidpur, Taki, and Jalalpur]. In the district of Khulna, *A. ludlowii* occurs extensively almost throughout the southern part of the district in areas cleared of the natural mangrove forests. It has been found in various localities in the Sunderban area [Nallianalla, Kobodak, Supoti, Beniakhali, Cassiabad, Dangmani, Dakupi, Kalabogi, Chandpie,

* These records are based solely on the author's investigations in Bengal. The specimens from the localities here recorded are preserved in the collections of the Bengal Malaria Research Laboratory at Calcutta.

MAP 1



Sarankhola and villages on the Haida Khal], at Debhata on the left bank of the Ichamati [Basantapur] and at Mollahat in the northern part of Khulna district

In the district of Khulna the distribution of *A. ludlowi* is more general and extensive than in the two other districts mentioned above and is in marked contrast to the greatly localized and patchy occurrence of the species in Budge-Budge, Chengail and Shamnagar. The intensive breeding of *A. ludlowi* in small areas that one observes in the latter places and the absence of the species in adjoining areas even a few miles away from these foci seem to indicate that we have here the early stages of colonization by a species new to the area through random scattering. It seems probable that the isolated and often widely separated foci of *A. ludlowi* that occur on the banks of the Hooghly are the result of a comparatively recent colonization of the inland areas of the delta by this sea-coast species.

Some support to the view that *A. ludlowi* is new to these areas (Budge-Budge, Chengail and Shamnagar) is to be had from the fact that this species was not observed there previously. Many years ago, Major C. A. Gourlay, I.M.S., made some very extensive collections of anophelines from Shamnagar and he did not come across even a single specimen of this species. Dr R. B. Khambata carried out a malaria survey of Budge-Budge in 1920 and he did not observe any *A. ludlowi* then. In connection with a malaria survey of the Rajapur Basin in Howrah district which was carried out in 1926, a large number of mosquitoes were caught from Chengail and its vicinity and this species was not observed in that area at the time.

From the nature of the distribution of *Anopheles ludlowi* on the banks of the Hooghly and from the fact that this species was not observed in these areas previously it is not improbable that this anopheline has colonized these areas in recent years. There is further support to this view in the fact that these areas, which were previously reputed to be entirely healthy, have suddenly during the past few years become intensely malarious. Moreover, the affected areas, which correspond closely with the *ludlowi* breeding grounds, have suffered severely from malaria, whereas the surrounding areas still have a low malarial endemicity.

In Lower Bengal the general level of land is low and many areas are lower than the spring-tide level. The banks of tidal rivers are generally embanked to protect the land and the crops from the tides. The greater part of the lower reaches of the Hooghly is thus embanked. Such protected land has innumerable collections of water which are not affected by the tides. Should such water collections have that concentration of salt and the other factors that may be necessary for *A. ludlowi* breeding, conditions are apparently favourable for this species to establish itself there.

There is a considerable amount of river traffic coming up the river Hooghly from the Saugor Islands and the Sunderban to Budge-Budge, Calcutta and even further north and it is conjectured that country boats, which come from these

areas where *A ludlowi* is common, are largely responsible for the introduction of this mosquito into these inland areas. The author has observed (Iyengar, 1930, p 264) that boats anchoring in channels in the cleared areas of the Sunderban are invaded by large numbers of anopheline mosquitoes, including *A ludlowi*. These mosquitoes often stay on board for several days and they could easily get transported over long distances. Considering the volume of river traffic from the Sunderban area, it seems very likely that this has been the manner in which *A ludlowi* has obtained entry into areas in which it was previously absent. But in spite of such chance scattering of the species occurring all along the length of the lower reaches of the Hooghly, we find this species only in a few isolated spots. The evident explanation of this is that *A ludlowi* has established itself only in those riverside areas where the water collections are of a suitable salinity as at Chengail and Budge-Budge, whereas in areas where such conditions do not exist, it has failed to do so.

Railway trains also seem to be of importance in transporting adult *Anopheles ludlowi* mosquitoes. In 1926, a specimen of *A ludlowi* was caught in a trap placed close to Sonarpur Railway station (13.3.26, P. Sur and M O T Iyengar coll.) and careful search showed that this species does not occur anywhere in the vicinity of Sonarpur. The only possible explanation seems to be that the mosquito was transported by a railway train from Port Canning where *A ludlowi* is not uncommon, to Sonarpur, the distance being less than 20 miles. Numerous trains run daily between these two places and this seems to be the most likely explanation of the observation. The importance of railway trains in transporting these mosquitoes should not be minimized.

Anopheles ludlowi in Khulna district

The southern part of the district of Khulna known as the 'Sunderban' was at one time almost entirely covered by dense natural mangrove forests. This region which comprises numerous islands of various sizes and is traversed by a network of tidal channels, is still partly covered by the natural mangrove forests, while large tracts have been cleared of the forest and the land cultivated. The land being below high-tide level, embankments are constructed alongside all the tidal channels in the cleared area to protect the soil and the crops from the salt-water tides. The collections of water that occur on such protected land are not affected by the tides nor could there be any natural drainage into the tidal channels close by, owing to the embankments alongside these channels. The soil in these areas being impregnated with salt, all collections of water are saline or brackish, the degree of which varies according to the salinity of the soil. These collections of water are suitable for *Anopheles ludlowi* breeding and this species occurs extensively in these embanked and cleared areas, frequently in very large numbers. On the other hand, where the natural mangrove forests are intact, this

anopheline is entirely absent. The contrast between the conditions in areas still under forest and those in the cleared areas is indeed very striking. The natural forests here form a definite protection against *A. ludlowi* as was shown in a previous paper (Iyengar, 1930).

When we consider the different cleared areas in the district of Khulna, some local variations in the incidence of this species are noticeable. For instance, the central part of the district in the region of the river Kobodak was observed to have a heavy incidence of *A. ludlowi* while in the eastern portion of the district as well as in the western portion, its incidence diminishes perceptibly. The reasons for such variations are not yet understood, but it seems probable that the salinity of the water and of the soil have considerable bearing on these variations.

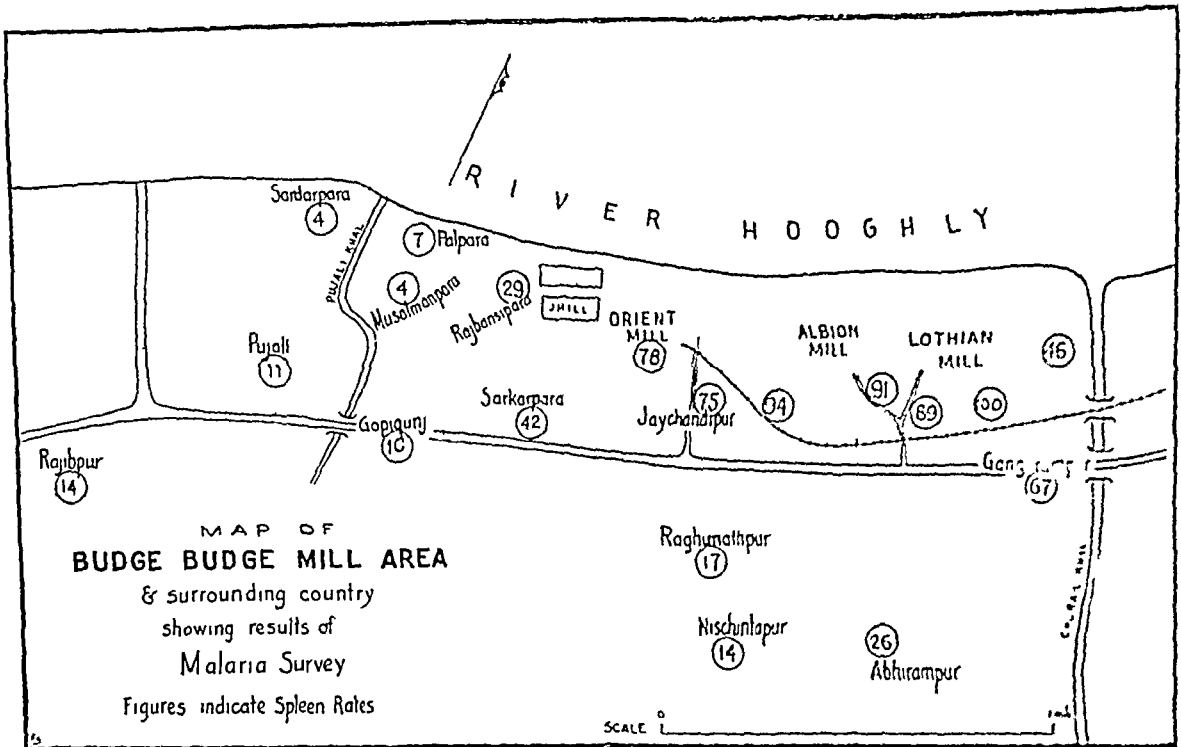
The epidemic of malaria at Budge-Budge during 1930

During the autumn of 1930, an epidemic of malaria of a severe type occurred in the industrial area south-west of Budge-Budge on the left bank of the river Hooghly. The epidemic was a very severe one and affected the entire resident population of the three mills, Lothian, Albion and Orient, and the rural areas adjoining them. Practically every one residing in this area was laid up with malaria and a large proportion of the population suffered from several severe attacks of malaria during the season. The medical officers of the mills reported that the infections were so heavy that large doses of quinine had to be administered to the patients before the fever could be brought under control. The incidence of sickness among the labour force as a result of this epidemic was so very high that it even affected the out-turn of these mills. In one of the mills situated in this area, a considerable fall in production occurred through invaliding and loss of efficiency among the labour force as a result of this epidemic of malaria.

This area was surveyed in October and November 1930. The spleen rates of children in the mill area and the area immediately adjoining it were very high, in some places the spleen rate was as high as 91 per cent and even as much as 100. The spleen rate among children of the Lothian Mill was 89.1 per cent, those of Albion and Orient Mills, 91.0 and 78.5 per cent respectively. In the rural areas adjoining these mills, spleen rates of 100, 94, 75, 67, 46 and 42 respectively were observed. Details of the results of examination of children in the mill area and the adjoining rural area is tabulated below. The relative position of the different localities surveyed and their respective spleen rates are marked on the sketch map of the area (see Map 2).

The very high spleen rates observed in the mill area and the adjoining rural areas indicate the severity of the infection here. The incidence of malaria, however, seems to be very largely localized, since in the rural areas at a distance of even a mile from this region, the spleen rates are considerably lower. As we

MAP 2



	Children examined	Children with enlarged spleen	Spleen rate	CLASSIFICATION OF SPLEEN					
				I 1	I 2	F 3	F 4	U	BL
1 Lothian Jute Mill	37	33	89 1	6	17	7	2	1	
2 Albion Jute Mill	78	71	91 0	13	29	18	7	3	1
3 Orient Jute Mill	14	11	78 5		2	8		1	.
4 Rural area immediately east of Lothian Mill	11	11	100 0	1	4	2	3	1	
5 Rural area immediately south of Albion Mill	17	16	94 1	3	6	3	2	1	1
6 Jaychandipur	48	36	75 0	17	11	6	2	1	1
7 Gangarampur	66	44	66 7	14	18	12			
8 Rural area east of Lothian Mill	48	22	45 8	10	10	2	.	.	.
9 Sarkarpara	69	29	42 0	16	12	.	1	.	
10 Rajbansipara	43	12	27 9	5	4	2	1		

proceed farther from the mill area the spleen rates diminish rapidly and at a distance of two or three miles, the spleen rates are below 20 per cent and often as low as 5 per cent. The following results of spleen census of children in the rural areas outside the affected region shows that the general incidence of malaria in this tract is quite low —

	Children examined	Children with enlarged spleen	Spleen rate	CLASSIFICATION OF SPLEEN					
				F1	F2	F3	F4	U	BU
11 Abhirampur	72	19	26.3	7	9	2			
12 Raghunathpur	57	10	17.5	4	6				
13 Nischintapur	64	9	14.1	2	7				
14 Gopiganj	41	4	9.7	1	3				
15 Palpara	44	3	6.8	2		1			
16 Kalipur, Musalman para	55	2	3.6	1	1				
17 Sardarpara	109	4	3.6	1	3				
18 Pujali	71	8	11.2	5	3				
19 Rajibpur	66	9	13.6	4	5				

The three villages Abhirampur, Raghunathpur and Nischintapur suffered from a definite increase of malaria during the last season, and the spleen rates of these three villages are decidedly higher than the general level of spleen rate in the area. The exceedingly high incidence of malaria in the mill area in contrast to the low malarial endemicity of the area surrounding it indicates that we had here an entirely localized epidemic due solely to local causes which did not appreciably affect the surrounding country.

Blood films (thin films) were taken from 183 adults and children residing in the Lothian and Albion Mills and examined for malaria parasites. Of these, 93 or 50.8 per cent were positive for malaria parasites in thin film examination.

Blood films examined	Positive for malaria parasites	Parasite rate
183	93	50.8

A parasite rate of 50.8 per cent in thin films is a fairly high figure. An analysis of the 93 blood films which were positive for malaria parasites is given below. All the three species of malaria parasites occurred here. The subtertian infection was the most frequent one in the area at the time and undoubtedly accounts for the severity of the epidemic.

Blood findings at Lothian and Albion Mills

Total positive films	Tertian	Sub tertian	Quartan	Tertian and Sub tertian	Tertian and Quartan
93	19	62	4	6	2

Taking them separately, the relative incidence of the three infections is as follows: sub-tertian 68, tertian 26 and quartan 6. Out of the 93 positive films, 19 of them showed gametocyte stages of the parasites, 12 had sub-tertian gametocytes and 7 had tertian gametocytes.

A survey of the *Anopheles* breeding places showed the following nine species breeding at the time: *A. subpictus* Grassi, *A. vagus* Don, *A. ludlowi* var *sundaica* Rodenwaldt, *A. fuliginosus* Giles, *A. varuna* Iyengar, *A. aconitus* Don, *A. ramsayi* Covell (*A. pseudojamesi* Str and Ch), *A. hyrcanus* var *nigerimus* Giles and *A. barbuostriis* Wulp. They were breeding in ditches and ponds in the area. In addition to these species found as larvæ, two more species, namely, *A. culicifacies* Giles and *A. pallidus* Theob., were caught as adults from houses. The two latter species occur in very small numbers only.

It was found that the incidence of adult *Anopheles* mosquitoes inside houses and lines was extremely heavy. Among adult mosquitoes caught in houses, *Anopheles ludlowi* was the most predominant species. Moreover, it was so numerous as to exceed in number all the other local species of *Anopheles* many times over. The huts and lines were teeming with adult *A. ludlowi* and large numbers of this mosquito could be caught without difficulty from these dwelling houses in a short time. Next to *A. ludlowi* in regard to numerical prevalence were *A. fuliginosus*, *A. varuna* and *A. aconitus*.

The breeding places of *A. ludlowi* were confined practically to the vicinity of the Albion and Lothian Mills. There are a few ponds in the Lothian Mill compound in which the breeding was particularly heavy. The incidence of *A. ludlowi* in houses was highest in the Lothian and Albion Mills lines and became noticeably less in places situated at some distance from this spot. A few specimens of this species were caught in villages about a mile away from this area, but their numbers were

small and they were obtained only after prolonged search. Apparently, as judged from conditions here, *A. ludlowi* prefers to stay close to its breeding place and does not proceed far from it.

The incidence of malaria in this area corresponds closely with the incidence of *A. ludlowi*. In areas close to the *A. ludlowi* breeding places, spleen rates are high—generally above 75 per cent, as we proceed farther from the *A. ludlowi* breeding area, the spleen rates diminish rapidly so that at a distance of a few miles, the spleen rates are below 15 per cent. These observations show the close relation between the incidence of malaria and the prevalence of *A. ludlowi*. This is but natural when we find the extreme susceptibility of *A. ludlowi* to infection with malaria parasites.

A large series of dissections of *A. ludlowi* mosquitoes was carried out by the author and it was found that this species has an abnormally high infection rate, a natural infection rate of 23.4 per cent was observed among 838 mosquitoes dissected. Such a heavy infection rate has not been observed in a large series of dissections with any species in India. Both from the point of view of numerical prevalence and from that of susceptibility to malarial infection, *A. ludlowi* is pre-eminently the important carrier mosquito in the area and is undoubtedly responsible for the severe epidemic of malaria that prevailed in this area. The following statement gives the results of the dissections carried out by the author of specimens collected from houses in the Lothian-Albion Mills area—

Results of dissections of Anopheles ludlowi

(Lothian-Albion Mills, October-November 1930)

MIDGUTS			SALIVARY GLANDS			TOTAL INFECTION		
Specimens examined	Specimens with oocysts	Oocyst rate	Specimens examined	Specimens with sporozoites	Sporozoite rate	Number of mosquitoes examined	Number with oocysts or sporozoites or both	Infection rate
836*	71	8.5	834*	169	20.3	838*	196	23.4

* The total number of mosquitoes dissected is 838, in the case of two specimens the midguts got damaged, and in four others the salivary glands were lost. This accounts for the difference in the number of specimens of midguts and of salivary glands examined.

An infection rate of 23.4 per cent is an extremely high figure, especially in such a large series of dissections as the present instance. There are only two previous

records* on the infectivity of *A. ludlowi* from India, both of which are from the Andaman Islands. Christophers (1912) found midgut infections in two out of 53 dissected (infection rate of 3·7 per cent), and Covell (1927) found gut infection in one and a salivary gland infection in another out of a total of 98 dissected (infection rate of 2·0 per cent). The exceedingly high natural infection rate now observed among *A. ludlowi* mosquitoes in the Lothian and Albion Mills in Budge-Budge shows the high susceptibility to infection of this species and also reflects on the epidemic conditions that prevailed there at the time.

The infectivity of the carrier species tends to be very much higher under conditions of epidemic malaria than under endemic or static conditions. In the former instance, the general parasite rate among the human population is very much higher than in endemic areas. In addition to this, owing to the fact that both adults as well as children are affected during an epidemic, adults are equally infective to *Anopheles* as children. In endemic areas on the other hand, it is only children that are largely affected, the adults having developed a comparative immunity, and as such, children form the main source of infection to the *anopheles*, while adults are generally not infective to them. As Swellengrebel and Swellengrebel de Graaf (1920) pointed out, 'In regions of epidemic malaria the number of carriers from which anophelines can acquire infection is much greater because of the many infected children and adults. In endemic regions, the number of carriers is less, these being for the most part children alone.' Swellengrebel and Swellengrebel de Graaf (1920) have shown how both the parasite rate and the gametocyte carrier rate are high during epidemic conditions while under endemic conditions they are both low, even though the spleen rates of children may be equally high in either case. They pointed out that gametocyte carriers are more numerous and more potent under epidemic conditions than under endemic conditions. As a result, the natural infection rate of the carrier *anopheles* tends to be very high in epidemic areas, while in endemic areas the infection rate of the carrier species is low even though the spleen rate among children may be high. During epidemic conditions, the infection rate among the population, the intensity of infestation and the proportion of potent carriers are all high as compared with those under endemic conditions.

The dissections of *A. ludlowi* mentioned previously are of specimens collected from the hives in the Lothian and Albion Mills where the spleen rates among children were very high, i.e., 89 and 91 per cent respectively. In addition to these, specimens of *A. ludlowi* were collected from two adjoining rural areas, Abhirampur and Sarkarpara, where the spleen rates were comparatively lower, namely 26 and

* The reported finding of one infected specimen of '*A. ludlowi*' by Horne in Madras City, recorded by Hodgson (1914, *Ind Jour Med Res*, I, 4), is omitted here, as it seems very doubtful if it actually refers to *A. ludlowi*. This species has not been observed by any other of the various workers in Madras (see foot note on page 499, ante).

42 per cent respectively. These were dissected and the results of the dissections are detailed below. The number of specimens obtained in these two places has been small owing to the sparsity of the species there. A natural infection rate of 26.6 per cent was observed among 15 specimens of *A. ludlowi* dissected from these two places.

Dissections of A. ludlowi from Abhinampur and Sarkarpura near Budge-Budge
(16th to 23rd November, 1930)

MIDGUTS			SALIVARY GLANDS			TOTAL INFECTION		
Number examined	Number with oocysts	Oocyst rate	Number examined	Number with sporozoites	Sporozoite rate	Number of mosquitoes examined	Number with oocysts or sporozoites or both	Infection rate
15	4	26.6	15	1	6.6	15	4	26.6

Hitherto we have been discussing the finding of naturally infected *Anopheles ludlowi* in this area. At about the same time when this work was in progress, specimens of *Anopheles ludlowi* bred from larvæ from a pond in the Lothian Mill were experimentally fed on patients with gametocytes in their blood. The mosquitoes were given a single infective feed after which they were kept on raisins and water. The results of three such infection experiments are given below—

Experiment I *Anopheles ludlowi* bred out of larvæ, fed on case No 10785 whose blood showed *Plasmodium vivax* gametocytes. Five mosquitoes fed on 12th November, 1930. Number that survived on 22nd November,—one, which was dissected on that date.

Result of the dissections —

Gut	+	Gland	+	Total	+
1	0	1	0	1	0

Experiment II *Anopheles ludlowi* bred from larvæ, fed on case No 11036 with gametocytes of *P. malariae* in his blood. Date of feed 15th November, 1930. Number that fed—ten. Number that survived on the 4th December—six. Date of dissection—4th December.

Result of the dissections —

Gut	+	Gland	+	Total	+
6	0	6	1	6	1

Experiment III *Anopheles ludlowi* bred from larvæ, fed on case No 11210 with gametocytes of *P falciparum* in his blood Date of feed, 19th November, 1930, number that fed—two Number that survived on the 4th December—one Date of dissection, 4th December

Result of the dissections —

Gut	+	Gland	+	Total	+
1	1	1	0	1	1

The series of infection experiments described above is too small to draw any definite conclusions on the comparative degree of susceptibility of *Anopheles ludlowi* to the three species of parasites While it is seen that *A ludlowi* is susceptible to experimental infection with *P malariae** and with *P falciparum*, in both of which the infection had reached the sporozoite stage in the salivary glands, the negative result in the case of the *P vivax* experiment denotes nothing as only one specimen was dissected

We have so far discussed the infectivity of *A ludlowi* in Budge-Budge under natural conditions and by experimental infection Specimens of other species of *Anopheles* were also collected from houses in the mill area during the same period and these were dissected The results of the dissections are tabulated below

Results of dissections of anophelines other than A ludlowi
(Lothian-Albion Mills, November 1930)

Species of Anopheles	MIDGUTS		SALIVARY GLANDS		TOTAL INFECTION	
	Number examined	Number with oocysts	Number examined	Number with sporozoites	Number examined	Number infected
<i>A fuliginosus</i>	34	0	34	1	34	1
<i>A hyrcanus</i>	3	0	3	0	3	0
<i>A culicifacies</i>	2	1	2	0	2	1
<i>A aconitus</i>	13	0	13	0	13	0
<i>A varuna</i>	6	0	6	0	6	0
<i>A pallidus</i>	1	0	1	0	1	0

* In view of the difficulty usually experienced in obtaining a sporozoite infection of the salivary glands with quartan parasites (*vide* James, S P, *Trans Roy Soc Trop Med Hyg*, XXIV, 5, p 482, 1931, March), the finding of one specimen of *A ludlowi* infected to the sporozoite stage of the quartan parasite is interesting The author has also been able to obtain sporozoite infections with *P malariae* in experimentally infected *A stephensi* and *A fuliginosus* in Calcutta These results will be embodied in a separate communication

The finding of naturally infected specimens of *Anopheles fuliginosus* and *A. culicifacies* in this area is interesting and shows that although *A. ludlowii* played the most important part in the epidemic of malaria that prevailed in Budge-Budge, there were also other species of *Anopheles* that were concerned in the malaria transmission. The negative findings in regard to the other species are not of a sufficiently large number to be of much significance.

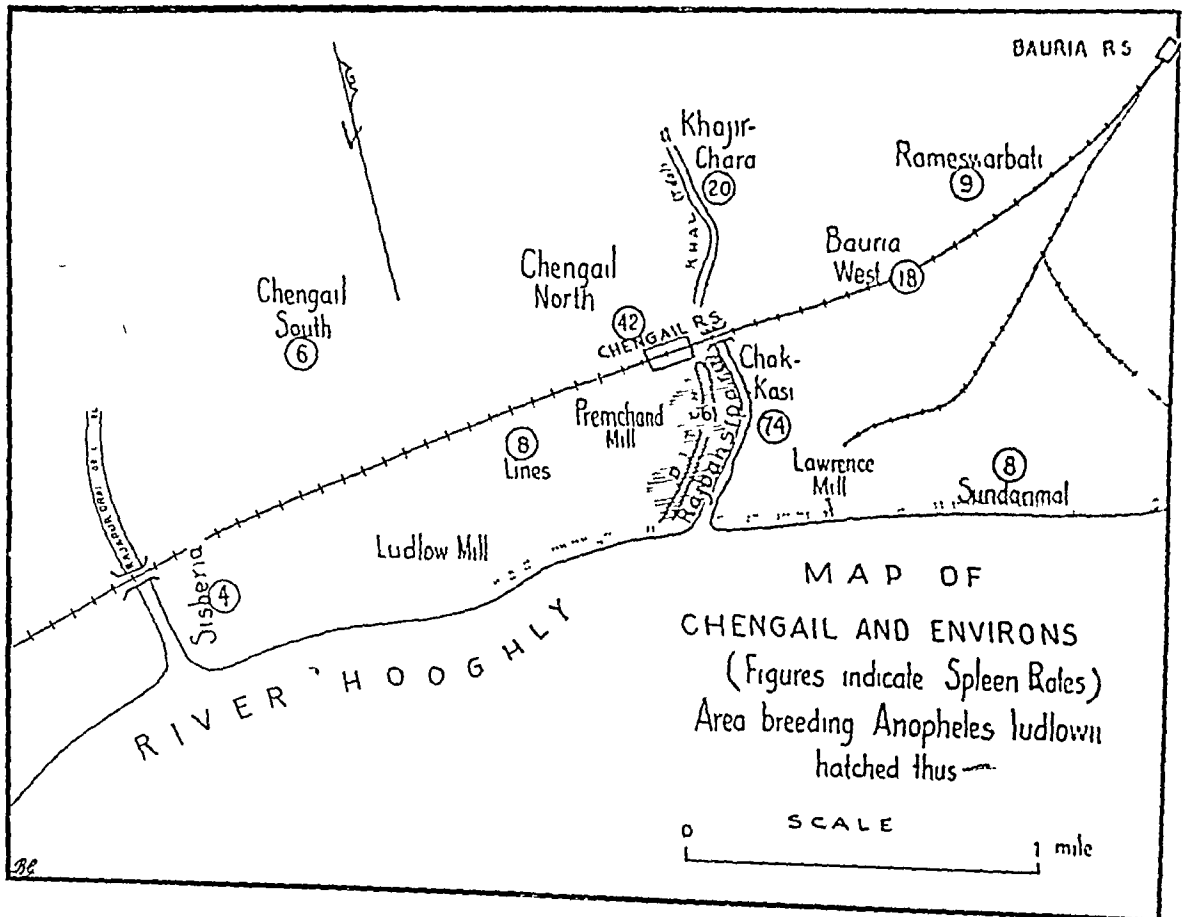
***Anopheles ludlowii* at Chengail, Howrah district**

When investigations into the epidemic of malaria at Budge-Budge were in progress, reports were received of an increase in the incidence of malaria among mill workers at the Ludlow Jute Mill at Chengail in Howrah District on the right bank of the Hooghly, a few miles down the river below Budge-Budge. An investigation was set on foot soon after receipt of the information. The medical officers of the Ludlow Mill informed the writer that, since September 1930, there has been a decidedly higher incidence of malaria cases among the labour force as compared with figures for the same period of the previous years and also that the individual cases of sickness were of a more persistent type requiring a longer course of treatment than ordinarily.

A malaria survey of the Ludlow Mill area and of the surrounding country was carried out during November-December 1930 and it was observed that the general incidence of malaria in the area was low except in a small area to the east of the Ludlow Mill. A sketch map of the area surveyed is reproduced here (see Map 3), and the spleen rates of the different localities are indicated on it. It will be observed that the spleen rates of Sisberia, Rameswarbati, Sundarimal and South Chengail are all below 10 per cent, and it is only in a small area including Rajbansipara, Chak Kasi and the small area surrounding it that the spleen rates are higher. Rajbansipara and Chak Kasi are two adjoining areas situated close to the river Hooghly to the east of the Premchand Mill and these two places have the highest spleen rates recorded in the area, namely, 61 and 74 per cent respectively. To the north of these, the spleen rates are lower. North Chengail which is nearest to this spot has a spleen rate of 42, Paschim Bauria and Kajirchora have still lower spleen rates, i.e., 18 and 20. Farther away from this area, the spleen rates diminish rapidly and are generally below 10. In Rajbansipara and Chak Kasi, there occurred an epidemic of malaria of moderate severity which affected a large proportion of the inhabitants. It appears that several persons in every house were laid up with attacks of malaria during the autumn of 1930. Very few persons escaped sickness, but the infection was not as severe as that observed at Budge-Budge. This area has been considered to be a very healthy one, and this view is supported by the low spleen rates of villages away from the affected zone. This has been the first year when there was such a severe outbreak of malaria in

which a large proportion of the population not only of Rajbansipara and Chak Kasi, but also of North Chengail suffered from attacks of malaria

MAP 3



Malaria survey of the Chengail area

	Children examined	Children with enlarged spleen	Spleen rate	CLASSIFICATION OF SPLEEN					
				F1	F2	F3	F4	U	BU
1 Ludlow Mill lines	212								
2 Rajbansipara	28	17	80	9	8				
3 Chak Kasi	19	17	60.7	10	5	2			
4 Bauria, West	50	14	73.7	5	8		1		
5 Chengail, North	59	9	18.0	4	5				
6 Kajrechara	50	25	42.4	10	10	1			
7 Chengail, South	48	10	20.0	6	4				
8 Sisberia	70	3	6.2	2	1				
9 Rameswarbati	64	3	4.2	2	1				
10 Sundarimal	36	6	9.4	4	2				
		3	8.3	2	1				

J, MR

A survey of the *Anopheles* breeding places was carried out simultaneously with the examination of children for enlarged spleen. The following ten species of *Anopheles* occur here: *A. subpictus*, *A. vagus*, *A. barbinotus*, *A. hyrcanus* var. *nigerrimus*, *A. fuliginosus*, *A. pallidus*, *A. varuna*, *A. aconitus*, *A. ramsayi*, and *A. ludlowi* var. *sundarica*. These species with the exception of *A. ludlowi* are distributed more or less generally over the whole area. *Anopheles ludlowi* was restricted to the Premchand Mill area and Rajbansipara. In this locality, there are several ponds and ditches holding brackish water which were breeding *A. ludlowi*. The *ludlowi* breeding area is indicated on the sketch map (see Map 3). The highest incidence of adult *A. ludlowi* mosquitoes was in the vicinity of Premchand Mill and Rajbansipara, localities very close to the breeding ground of the species. A small number of adult specimens were caught in the lines of Ludlow Mill which is less than half a mile from this area, a few were obtained from Bauria which is about a mile away, and one specimen from Sisberia which is a mile and a half away. The heavy incidence of *Anopheles ludlowi* adults in the immediate vicinity of its breeding place and its sparseness at a distance of even a mile from this spot seem to indicate that *A. ludlowi* prefers to stay close to its breeding place. A similar observation was made in the Lothian-Albion Mills area in Budge-Budge where the incidence of *A. ludlowi* was very heavy in the vicinity of the breeding place and diminished markedly within even a mile from the breeding area.

Although there are several carrier species in this locality, *A. ludlowi* seems to be the most important one as the incidence of malaria corresponds closely with the *A. ludlowi* breeding area. Places close to the breeding area of this species, such as Rajbansipara and Chak Kasi, have high spleen rates. Those farther away show lower spleen rates and localities over a mile away from this area have very little malaria (see Map 3). We observe here a very interesting correlation between the spleen rate and the proximity of the *A. ludlowi* breeding area.

The coolie lines of Ludlow Mill which are not far from this *A. ludlowi* area has a comparatively low spleen rate of 8 per cent. But when we take into consideration the great density of population in these lines and the very efficient and well organized medical facilities available to the workers of this mill, both of which factors tend to lower the spleen rate considerably, a spleen rate of 8 per cent is not as low as it looks. There has been a definite increase in the sickness due to malaria in the lines. As, however, the epidemic that occurred in Rajbansipara and Chak Kasi during 1930 was not so extensive or so severe as the one that occurred in Budge-Budge. The affected localities were the immediate vicinity of the *A. ludlowi* area.

There is more than mere circumstantial evidence supporting the view that *A. ludlowi* is the main cause of the trouble at Chengail. A natural infection rate of 4.2 per cent was observed among 71 specimens of *A. ludlowi* dissected. The

specimens were collected from Rajbansipara in November 1930 and the results of the dissections are detailed below —

Results of dissections of A ludlowi from Chengail
(November 1930)

Date of collection	Date of dissection	MIDGUTS.			SALIVARY GLANDS			TOTAL INFECTION		
		Number examined	Number with oocysts	Oocyst rate	Number examined	Number with sporozoites	Sporozoite rate	Number examined	Number infected	Infection rate
17-11-30	28-11-30	15	0	0	15	0	0	15	0	0
17-11-30	29-11-30	31	0	0	31	2	6.5	31	2	6.5
26-11-30	6-12-30	25	0	0	25	1	4.0	25	1	4.0
TOTAL		71	0	0	71	3	4.2	71	3	4.2

The close correlation between the incidence of malaria and the proximity of breeding places of *A ludlowi*, and the finding of an infection rate of 4.2 per cent among specimens caught in nature indicate that this species is the important transmitter here. An infection rate of 4.2 per cent, although it is not so high as the figure for the same species that was observed at Budge-Budge, is quite high by itself. The difference in the infectivity rates of *A ludlowi* in Chengail and in Budge-Budge is probably due to two causes. The specimens from Chengail were caught late in the season, nearly a fortnight later than those collected at Budge-Budge. But the more important reason appears to be due to the fact that at Budge-Budge the infection among the human population was the more extensive and severe one as compared with conditions at Chengail. A comparison of the spleen rates of the two areas brings out strikingly the intense and widespread infection at Budge-Budge in contrast to the less severe and greatly restricted incidence of the infection at Chengail. In Chengail we have the conditions that prevail during the early stages in the progressive development of epidemic conditions, while at Budge-Budge the epidemic is in full swing.

Anopheles ludlowii in Shamnagar, 24-Periganas district

At Shamnagar which is 19 miles north of Calcutta, on the left bank of the Hooghly, there was much sickness due to malaria during 1929 and 1930. During the autumn of 1930 some blood films were obtained from cases of malaria in this place and a heavy incidence of *P. vivax* infections was observed. In many of the houses at Shamnagar, several persons were laid up with malaria. No one thought that there was anything unusual in the increase of malaria at Shamnagar, until one day in December 1930, Dr H. Sarkar, who resides there, collected a single specimen of *Anopheles ludlowii* from inside a mosquito curtain used by his children who were all down with malaria. This specimen was seen by the writer and subsequently a survey party was sent round to look for adult and larval specimens of *A. ludlowii* from the vicinity of Shamnagar. Over 20 specimens of *A. ludlowii* have since been obtained from this place, but the breeding places have not yet been discovered. In January 1931 when the survey parties went to Shamnagar, most of the breeding places were dry and the breeding season was evidently over. It is proposed to carry out a detailed survey of the area round Shamnagar during the ensuing wet season.

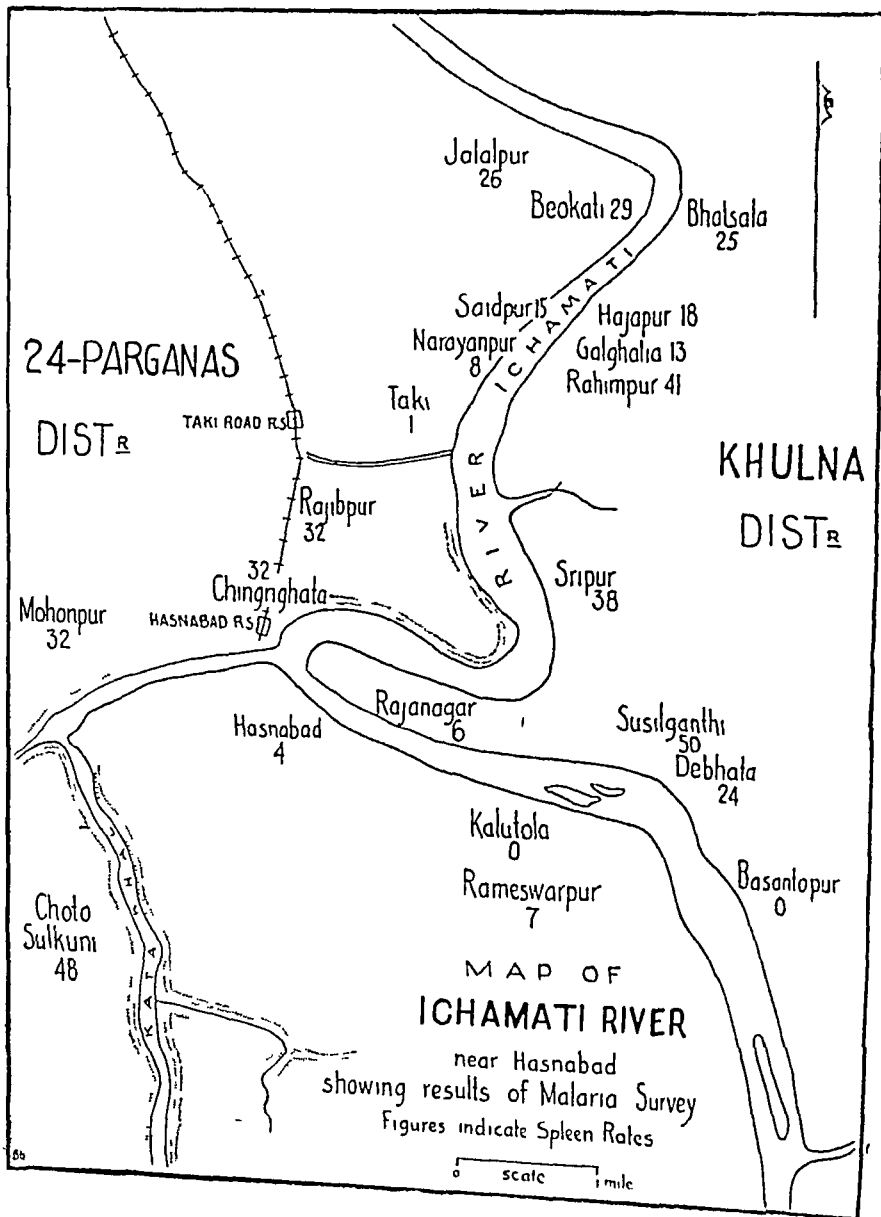
The finding of *A. ludlowii* in Shamnagar and the reported heavy increase in the incidence of malaria in that locality seem to indicate that at Shamnagar there has been an epidemic of malaria of a mild type in which possibly *A. ludlowii* was an important carrier.

Anopheles ludlowii on the banks of the Ichamati

The river Ichamati which separates the two districts, 24-Periganas and Khulna, is a tidal river, the banks of which are embanked in some places while other portions are not so embanked. In many of the villages on the banks of this tidal river, *Anopheles ludlowii* has been observed to occur but its incidence is not heavy. This species appears to have a general distribution over the country here. In addition to *A. ludlowii*, other species of *Anopheles* have been observed here, namely, *A. fuliginosus*, *hycanoides* var. *negerrimus*, *A. aconitus*, *A. varuna*, *A. pseudojamesi* (*A. ramsayi*) and *A. vagus*. To study the incidence of malaria in this area, a survey of the villages on either banks of the Ichamati in the region of Hasnabad and Debhata was carried out. The spleen rates ranged between 0 and 50 per cent, the area surveyed and the results of the spleen rates are marked on the sketch map reproduced here (see Map 4). The details of the result of the examination of children in this area are given in the Appendix. Although adult specimens of *Anopheles ludlowii* were collected from villages here, the breeding places and the monsoon conditions in this area could not be studied as the survey was carried out during last winter. The incidence of malaria is very varying, but

one point that is strikingly clear here is that malaria is endemic and not epidemic as was observed in Budge-Budge and other places discussed above. The distribution of *A. ludlowi* is more general. As this area is so close to Khulna district in which *A. ludlowi* is fairly common, it seems likely that it has been in association with *A. ludlowi* for quite a long time and we do not therefore observe the severe reactions that follow the new introduction of a virulent carrier species into an area which did not have it before.

MAP 4



Salinity of water in relation to A. ludlowi breeding

The salinity content of all *Anopheles* breeding places in Budge-Budge and Chengail was determined with a view to understand the degree of salinity of the water collections and to see if any relation exists between the salt concentration of the water and the species of *Anopheles* breeding in it. The main object however was to determine the salt concentrations in which *A. ludlowi* occurred in these two areas. Rodenwaldt and Essed (1925) made some extensive observations on the salt concentrations in relation to *A. ludlowi* breeding in the Dutch East Indies. Christophers (1912) made some observations on the salinity of water in the Andamans. No extensive work on the relation of the salinity of the water to *A. ludlowi* breeding has been made in India.

The salinity determinations were made possible through the courtesy of Mr N K Chatterjee and Mr S C Ray, chemists in the Bengal Public Health Laboratory, who carried out this work. The work was carried out during last December and January. The salinity concentrations of 237 samples of water from breeding places in which *Anopheles* larvæ were found were determined. At the same time, the species of *Anopheles* found in the ponds were also recorded. Larval determinations were made in all cases excepting *A. subpictus* and *A. ludlowi* which were bred out for determination.

The relative frequency of the different degrees of salinity of the water collections in Chengail and in Budge-Budge is seen from the following analysis of the determinations —

Sodium chloride milligrams per litre of water	0—100 mg per litre	Per cent	100—1,000 mg per litre	Per cent	1,000—1,500 mg per litre	Per cent	1,500—2,000 mg per litre	Per cent	2,000—2,500 mg per litre	Per cent	2,500—3,000 mg per litre	Per cent
Budge Budge area	64	53.3	31	25.8	15	12.5	4	3.3	3	4.2	1	0.8
Chengail area	89	74.4	19	16.2	8	6.8	1	0.8		0		0
TOTAL	153	64.6	50	21.1	23	9.7	5	2.1	5	2.1	1	0.8

At Budge-Budge, the ponds have a decidedly higher average salinity than those at Chengail. For instance, 21 per cent of the ponds in Budge-Budge had a salinity of more than 1,000 milligrams of sodium chloride per litre of water, whereas at Chengail only 7.6 per cent of them had more than 1,000 mg per litre. The more extensive prevalence of *Anopheles ludlowi* at Budge-Budge as compared with that at Chengail is largely due to the greater prevalence in the former area of ponds with a salinity figure higher than 1,000 mg per litre, as will be seen later.

The relative frequency of the different species of *Anopheles* in water of different salt concentrations has been worked out and the results are of interest. Some of the species show a positive correlation with the increase of salinity and some a negative one. The protocols of these observations are tabulated below —

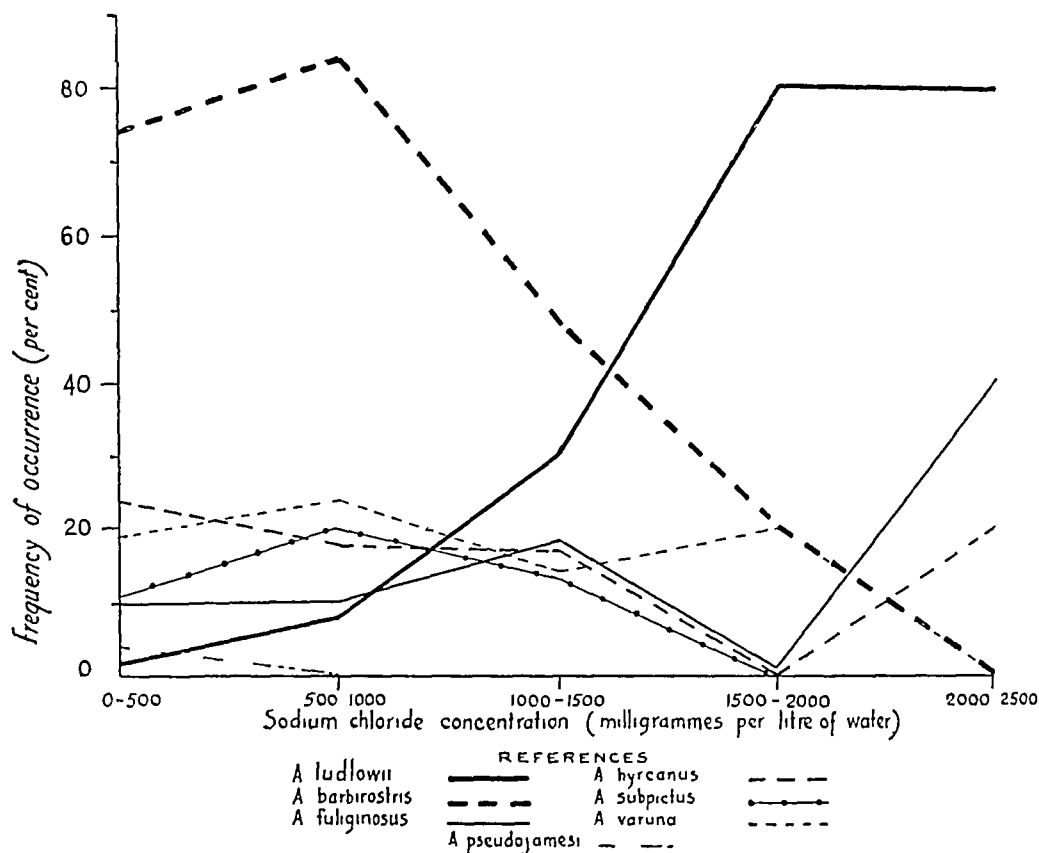
Sodium chloride mg per litre	0-500	500-1,000	1,000-1,500	1,500-2,000	2,000-2,500	2,500-3,000
Observations	153	50	23	5	5	1
<i>A. barbirostris</i>	113 73.9	12 84.0	11 17.8	1 20.0	0	0
<i>A. sinensis</i>	36 23.5	9 18.0	1 17.4	0	1 20.0	0
<i>A. fuliginosus</i>	16 10.5	5 10.0	4 17.4	0	2 40.0	1 100.0
<i>A. pseudojamesi</i>	5 3.3	0	0	0	0	0
<i>A. vagus</i>	0	1 2.0	1 4.3	0	1 20.0	0
<i>A. subpictus</i>	17 11.1	10 20.0	3 13.0	0	0	0
<i>A. varuna</i>	29 19.0	12 24.0	3 13.0	1 20.0	0	0
<i>A. aconitus</i>	19 12.4	3 6.0	1 4.3	0	0	0
<i>A. ludlowi</i>	3 2.0	4 8.0	7 30.4	4 80.0	4 80.0	0

Anopheles barbirostris occurs in 74 per cent of the breeding places with 0 to 500 mg of sodium chloride per litre, it increases to 84 per cent in waters with 500—1,000 of salt per litre. From this point, it drops rapidly as the salinity increases (see Chart). *Anopheles hyrcanus* occurs in 23.5 per cent of the breeding places with 0-500 mg per litre, in the next grade of salinity, namely, 500-1,000 milligrams of sodium chloride per litre, it is found in 18 per cent of the breeding places. It has a similar frequency (17.4 per cent) in the next higher grade of salinity and then drops to nothing in the grade of 1,500-2,000 milligrams per litre. The 20 per cent figure in the 2,000-2,500 mg per litre grade is based on a single finding out of five observations and does not have much significance. *Anopheles fuliginosus* has nearly the same frequency in all grades of salinity observed here. It has been observed to breed in water with a sodium chloride content of as much as 2,700 milligrams per litre of water and it seems to be quite tolerant to blackish water.

Anopheles ramsayi seems to be particularly susceptible to salinity concentrations. It occurs only in the 0-500 milligrams per litre grade and it is entirely absent in any of the higher concentrations. *Anopheles aconitus* starts with a 12 per cent frequency in the first grade and drops gradually to zero as the salinity

increases *Anopheles varuna* starts with a 19 per cent frequency in the first grade, rises to 24 per cent in the second grade and then declines *A. subpictus* also behaves similarly The number of *A. vagus* observed here is too small to be of value

CHART



Anopheles ludlowi is the only species which shows a positive correlation with the salinity. It starts with a 2 per cent frequency in the first grade, rises to 8 and 30 per cent in the second and third grades. In the next two grades, namely, 1,500–2,000 and 2,000–2,500 mg per litre, *A. ludlowi* occurs in 80 per cent of the breeding places. These results show that the optimum salinity for the breeding of *A. ludlowi* is between 1,500 and 2,500 milligrammes per litre of water. In the series of observations made here, the highest salinity figure obtained was 2,700 mg per litre and as such the range of observations is not large enough to fix a correct optimum figure with any degree of certainty.

In addition to the salinity factor, another factor seems to have a considerable bearing on the breeding of *A. ludlowi* and that is the presence of organic matter in the water. The presence of organic matter was determined by tests for nitrites in the water. The amount of nitrites in the present series of observations is not

very large but a good number of them showed minute traces of nitrites. In going through the results of these determinations, it was observed that in every one of the ponds in which a salinity of more than 1,000 milligrams of sodium chloride per litre of water occurred in combination with the presence of a trace of nitrites, *Anopheles ludlowi* was invariably found to breed in it. Where the salinity was more than 1,000, but there was no trace of any nitrites, *A. ludlowi* occurred only very rarely.

The results of these observations in Bengal differ largely from those of Rodenwaldt and Essed (1925) in the Dutch East Indies where it was found that a salt concentration of 1.2 to 1.8 per cent was most favourable for *A. ludlowi* breeding in that country and that this mosquito would breed in water with as much salt as even 3 per cent. The optimum salinity for *A. ludlowi* observed in Budge-Budge and Chengail is indeed very low as compared with those observed in the Dutch East Indies, in fact it is just a tenth of the latter figures. Christophers' (1912) figure of 0.4 per cent salt in the breeding places of *A. ludlowi* in the Andamans is much nearer the Bengal figure. Rodenwaldt and Essed (1925) also observed that in the Dutch East Indies, *A. ludlowi* would breed quite well in putrid water. Although in regard to the present limited observations in Bengal, *A. ludlowi* has not been found to breed in putrid water, there is no doubt that it breeds in water with some organic contamination. The results show that *Anopheles ludlowi* prefers a small amount of contamination in the water, although not such an extent as that observed in the Dutch East Indies. A combination of the salinity factor with the presence of a small amount of organic contamination seems to be the optimum condition for the breeding of *A. ludlowi* in this area, as seen from the fact that although the salt concentration was above 1,000 mg per litre, *A. ludlowi* did not breed in it unless the sample showed the presence of nitrites.

The author is much indebted to Dr C. A. Bentley and Dr S. N. Sur for invaluable help received during the course of these investigations and to Dr R. B. Khambata for his help in connection with the estimation of salinity of the water samples.

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APPENDIX

Malania survey of the banks of the Ichamati near Hasnabad

District	Village	Children examined	Children with enlarged spleen	Spleen rate	CLASSIFICATION OF SPLEEN					
					F1	F2	F3	F4	U	BU
24-PERGANAS	Chingrighata	19	6	31.5	1	3		2		.
	Rajibpur	52	16	30.7	6	4	3		2	1
	Taki	74	1	1.3		1				
	Narayanpur	25	2	8.0	1		1			
	Saidpur	53	8	15.1		6	2			
	Beokati	24	7	29.1		4	2		1	
	Jalalpur	40	10	25.0	2	5	2	1		
	Hasnabad	54	2	3.7	1	1				
	Kalutola	50	0	0.0						
	Rameswarpur	46	3	6.5		1	1		1	
	Choto Sulkuni	23	11	47.9	5	3	1	1		1
	Mohonpur	22	7	31.8	2	2	3			
KHULNA	Debhata	76	18	23.6	4	10	2	1	1	
	Susilganthi	24	12	50.0	3	5	1	2	1	
	Sripur Town	42	16	38.1	3	4	4	5		
	Galghalia	30	4	13.3	1	2	1			
	Hajapur	28	5	17.8		5				
	Bhatsala	24	6	25.0	1	3	2			
	Rajanagar	54	3	5.5	1	1	1			.
	Rahimpur	22	9	40.9		6	1	2		
	Basantopur	33	0	0.0						



Fig 1



Fig 2

EXPLANATION OF PLATE XXIX

Photographs of afforested areas in the Sunderban, covered with dense mangrove forests

- Fig 1 General view of a tidal channel passing through the Sunderban forest area
„ 2 Closer view of the vegetation The level of land is very low and the tides rise so high as to cover a large portion of the land surface during spring tides

EXPLANATION OF PLATE XXX

- Fig 1 Photograph of a village in the cleared area of the Sunderban showing the high embankment on the right constructed to protect the land from the tides. The river is to the right side of the embankment. *Anopheles ludlowi* breeds heavily on such cleared and protected areas.
- „ 2 Photograph of another cleared area in the Sunderban. To the left is Harda Khal, a tidal channel and on the other side of Harda Khal the land is still under the natural mangrove forests. The embankment in the middle of the picture protects the cleared land from the tides. The contrast between the two sides of the tidal channel in regard to the prevalence and breeding of *Anopheles ludlowi* is very striking. On the cleared area, the incidence of this mosquito is very heavy, whereas in the afforested areas, it is entirely absent.



Fig 1



Fig 2

THE RELATIVE VALUE OF THE OOCYST RATE AND THE SPOROZOITE RATE IN ANOPHELES

BY

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[Received for publication, June 8 1931]

IN recording the results of dissections of anopheline mosquitoes for malaria parasites, it is advantageous that records of each series of dissections are maintained in the following manner —

MIDGUT			SALIVARY GLANDS			TOTAL INFECTION		
Number examined	Number with oocysts	Oocyst rate	Number examined	Number with sporo zoites	Sporozoite rate	Number of mosquitoes examined	Number with oocysts or sporo zoites or both	Infection rate

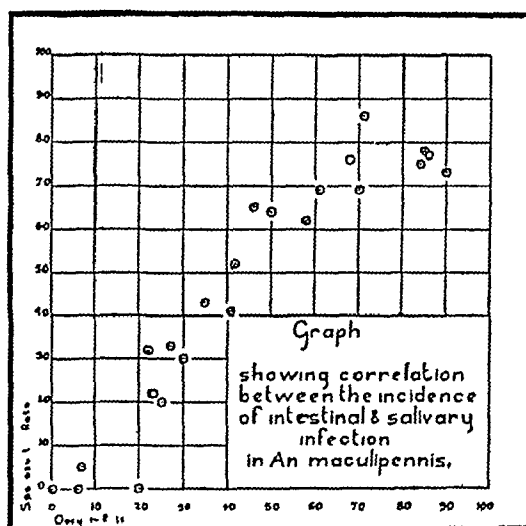
Such a record would give the oocyst rate, the sporozoite rate and the total infectivity rate separately for each series of observations. The total infectivity rate is a combination of the former two and is based on specimens observed to have either an oocyst infection of the gut, or a sporozoite infection of the salivary glands or both. In determining the natural infection rate of a species of *Anopheles*, many workers take this combination of oocyst infections and sporozoite infections. On the other hand, there are several workers who altogether overlook oocyst infections of the gut and place reliance solely on sporozoite infections of the salivary glands. They argue that there is no

certainly that all the oocysts found in the gut wall would mature and discharge sporozoites, they contend therefore that an infection rate which includes oocyst infections would not represent the transmitting capacity of the species. The latter school of workers would often examine only the salivary glands for the presence of sporozoites and would entirely ignore midgut infections. A further step in this direction is the evolution of the technique of Sergent and Sergent by which a smear of the body-fluid from the neck and anterior thoracic region of the mosquito is stained and examined for sporozoites.*

* On the other hand, Swellengrebel and De Buck (1931) maintain that midgut infection is a more accurate measure of the transmitting capacity of a species than salivary infection and they suggest that where circumstances do not allow the examination of both the gut and the gland, precedence should be given to the former.

This view is expressed in a recent paper by Swellengrebel and De Buck (Correlation between intestinal and salivary infection in *Anopheles maculipennis*, *Proc Konink Akad v Wetn*, Amsterdam, XXIV, 1, 1931, pp 183-185) which was seen by the writer after he sent the present paper to press. These authors have brought convincing evidence from the results of experimental infection of *A. maculipennis* with *Plasmodium vivax* to show the very close correlation between midgut infections and salivary gland infections in the mosquitoes. Out of 23 batches of experimentally infected mosquitoes, the mean sporozoite rate and the mean oocyst rate were observed to be 47 per cent and 45 per cent respectively. To quote from that paper, 'the close correlation between the two is plainly expressed in the accompanying Graph and is more accurately expressed by the formula $r = + 0.939 \pm 0.024$ '.

Their Graph is reproduced below.



(From Swellengrebel and De Buck, 1931)

For working out the infectivity of any species, Swellengrebel and De Buck maintain that although there is a close relation between the sporozoite rate and the oocyst rate, the former is not as accurate a

It is not justifiable in working out the infectivity of species of *Anopheles*, that oocyst infections of the gut should be altogether ignored. The author considers that in those instances in which gut infections are observed to occur in a species under natural conditions and no sporozoite infections of the salivary glands whatsoever, the infection rate based solely on oocyst infection may not represent the transmitting capacity of the species in relation to the period of observation. It is evident that, in those circumstances, certain factors (which are not yet well understood) prevent the development of the oocysts. Under such conditions, it seems unlikely that the oocysts observed in the gut would develop and mature to produce a sporozoite infection of the salivary glands. But where both gut infections and gland infections occur side by side often in the same individual specimen, under such circumstances, oocyst infections should certainly be included in the total infectivity rate in order to appraise the transmitting capacity of the species. Every oocyst then observed in the gut is a potential source of infection of the salivary glands, it is extremely unlikely that when other oocysts have matured and produced the infection of the salivary glands with sporozoites, the oocysts then occurring in the midgut would not similarly complete their development under normal course of events.

INCIDENCE OF OOCYST AND SPOROZOITE INFECTIONS IN *Anopheles ludlowi*

The view expressed above is supported by the results of four series of dissections of specimens of *Anopheles ludlowi* var. *sundarica* Rodenwaldt, all of which were collected from the Lothian-Albion Mill Lines near Budge Budge, 24-Perganas district in Lower Bengal. An epidemic of malaria of a severe type occurred in this area during the autumn of 1930 and a very high natural infection rate was observed among *A. ludlowi* mosquitoes. The four series of dissections detailed herein are of specimens collected from this locality at different periods between October 1930 and January 1931.

SERIES I

This series comprises the largest number of dissections of all the four series discussed herein. The specimens were collected between 28th October and 9th November, 1930.

measure as the latter. They point out that 'in various investigations on natural infection of *Anopheles*, the oocyst rate almost invariably surpasses the sporozoite rate, the relation between the two ranging from 1½ to 6 to 1, in *A. maculipennis* this relation is 5 to 1. They conclude that 'in *A. maculipennis* the incidence of intestinal infection allows of a more accurate estimate of the actual number of sporozoite carriers than does the incidence of salivary infection. At present there is no reason why this conclusion should not apply to other species likewise.'

Anopheles ludlowi dissections, Series I *

MIDGUTS			SALIVARY GLANDS			TOTAL INFECTION		
Number examined	Number with oocysts	Oocyst rate	Number examined	Number with sporozoites	Sporozoite rate	Number of mos quitoes examined	Number with oocysts or sporozoites or both	Infection rate
836	71	8.5	834	169	20.3	838	196	23.4

In this series the intensity of oocyst infections was heavy and oocysts in all stages of development were observed. The sporozoite infestation of the salivary glands was generally heavy, while in some the sporozoite infestation was poor.

SERIES II

This series comprises a comparatively smaller number of observations. The 54 specimens dissected in this series were collected on 7th December, 1930, a month later than those of Series I.

Anopheles ludlowi dissections, Series II

MIDGUTS			SALIVARY GLANDS			TOTAL INFECTION		
Number examined	Number with oocysts	Oocyst rate	Number examined	Number with sporozoites	Sporozoite rate	Number of mos quitoes examined	Number with oocysts or sporozoites or both	Infection rate
54	10	18.5	54	2	3.7	54	10	18.5

The total infection rate of this series is lower than that of Series I, the oocyst infestation was decidedly poorer and the individual oocysts were mostly of the half-developed stage. Very few oocysts were observed in any advanced stage of development. Only two out of the 54 specimens had a sporozoite infection of

* A summary of the results of Series I was published in connection with another investigation, vide Iyengar, 1931, *Ind Jour Med, Res*, Vol XIX, No 2, October, pp 499—524.

the salivary glands and even in these two specimens, the number of sporozoites occurring in the glands was found to be small and the sporozoite infestation was decidedly sparse

SERIES III

The 34 specimens comprising this series were collected from the same locality on 24th December, 1930, 17 days later than Series II. It was difficult to obtain a larger number of specimens for dissection at this time owing to the great sparsity of mosquitoes of this species. This sparsity was probably due largely to the intensive anti-mosquito measures that were pushed vigorously at the time and partly also to the adverse mosquito season. The results of these observations are detailed below

Anopheles ludlowi dissections, Series III

MIDGUTS			SALIVARY GLANDS			TOTAL INFECTION		
Number examined	Number with oocysts	Oocyst rate	Number examined	Number with sporozoites	Sporozoite rate	Number of mosquitoes examined	Number with oocysts or sporozoites or both	Infection rate
34	5	14.7	34	0	0	34	5	14.7

The total infectivity rate of this series is much lower than those of the two previous ones. All the infections observed in this series are oocyst infections of the gut, no sporozoite infections of the salivary glands were at all observed.

SERIES IV

The specimens comprising this series were collected from the same quarters three weeks later than those of Series III, namely on 14th January, 1931. The number of observations in this series is small, the incidence of adult *A. ludlowi* was extremely low at the time. The adverse season and the anti-mosquito measures that were carried out were probably responsible for this large reduction in its incidence. Only 16 specimens were obtained after a prolonged search. Although the number of observations in this series would, under ordinary circumstances, be considered too small to justify any broad conclusions, the results of this series of dissections, when considered in conjunction with those of the three previous series, are significant.

Anopheles ludlowi dissections, Series IV

MIDGUTS			SALIVARY GLANDS			TOTAL INFECTION		
Number examined	Number with oocysts	Oocyst rate	Number examined	Number with sporozoites	Sporozoite rate	Number of mos quitoes examined	Number with oocysts or sporo zoites or both	Infection rate
16	0	0	16	0	0	16	0	0

The entire absence of oocyst infections and of sporozoite infections in the specimens comprising this series is of interest

A consideration of all the four above-mentioned series of observations together conveys interesting information on the seasonal infectivity of *A. ludlowi* and the variations in the incidence of oocyst and sporozoite infections. The following is a summary of the results of the four series of dissections. As all these observations were carried out at the same place and under the same conditions, they are strictly comparable.

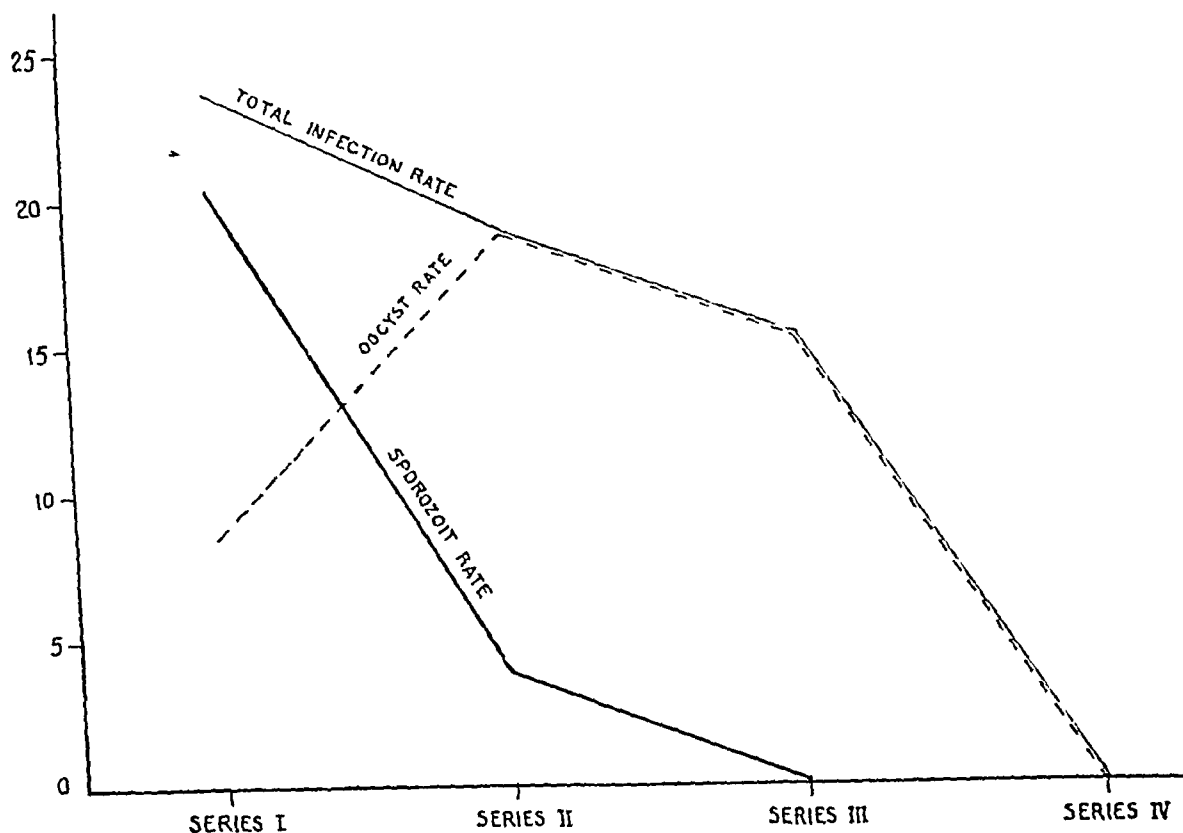
Summary of the observations, Series I to IV

	SERIES I	SERIES II	SERIES III	SERIES IV
Date of collection	28th October— 9th November	7th December	24th December	14th January
Total infectivity rate	23.4	18.5	14.7	0.0
Sporozoite rate	20.3	3.7	0.0	0.0
Oocyst rate	8.5	18.5	14.7	0.0

The infectivity rate of *A. ludlowi* is highest in Series I and it decreases rapidly in the subsequent series. It is 23.4 per cent in Series I, 18.5 in Series II, 14.7 in Series III and zero in Series IV (Chart 1). The fall in the infectivity of *Anopheles ludlowi* from the very high infection rate of 23.4 per cent to zero in the course of a few months appears to be due to the onset of the adverse season, namely, the winter. As these observations were carried out under identical conditions, on mosquitoes

of the same species collected from the same habitations in the Lothian-Albion Mill area, it is unlikely that there could be any explanation of this fall in the infectivity of *A. ludlowi* other than that of the change of season from autumn to winter.

CHART 1



The effect of the adverse season is also noticeable in regard to the sporozoite rates and the oocyst rates in the four series of observations. The sporozoite rate of Series I is very high, namely, 20.3 and it suddenly drops to 3.7 in Series II, in the last two series, it is zero (Chart 1). These observations indicate that the infections observed in Series I were the result of mosquito infection at a time when conditions were favourable for the development and maturing of oocysts in the mosquito and the production of a sporozoite infection of the salivary glands. The subsequent series of observations are in seasons during which it was increasingly difficult for oocysts to mature and produce sporozoites. In Series I, a high infectivity rate and a high sporozoite rate were observed. In Series II, which was carried out a month later, the infectivity rate was lower than that of Series I and the sporozoite rate suffered a big drop. This indicates that while conditions were getting to be

less favourable for the infection of the mosquito as indicated by the lower total infectivity rate, they were definitely adverse to the maturing of the oocysts and the production of sporozoites. In this series, most of the oocysts observed were of the half grown stage. The very sparse infestation of the salivary glands observed in two specimens in that series appears to be the remains of a previous sporozoite infection and it seems evident that there had been no recent production of sporozoites. In Series III, the total infectivity rate is lower still than in Series II, and the infection consisted entirely of gut infections. The presence of oocyst infections without corresponding sporozoite infections in this series denotes a state in which conditions are not unfavourable for the formation of oocysts, but that some factors prevent or delay the development and maturing of the oocysts. In Series IV, if we may rely on the comparatively smaller number of observations, conditions are apparently unfavourable even for the formation of oocysts.

In passing from Series I to Series IV, it seems evident that we pass from a period which was favourable for the development of oocysts and the production of sporozoite infections, to a transitional period when only oocyst infections were possible, and thence to a season when conditions were unsuitable even for the formation of oocysts. The fall in the total infection rate and in the sporozoite rate prove this contention.

It should, however, be observed that the oocyst rate does not exhibit a uniform fall from Series I to Series IV (Chart 1). In Series I, it is 8.3 and in Series II, it rises very suddenly to 18.5. From that point it falls to 14.7 in Series III and thence to zero in Series IV. This rise in the oocyst rate from 8.3 in Series I to 18.5 in Series II is indicative of the transitional period between a favourable season and an unfavourable season. This rise of the oocyst rate could be explained in the following manner —

Although there is a reduction in the total infectivity rate from 23.4 in Series I to 18.5 in Series II, the oocyst rate actually shows a rise from 8.3 to 18.5. This is due to the fact that while in Series I a large proportion of the oocysts were capable of maturing and bursting, such maturing of oocysts was greatly suppressed in Series II and the infections persisted in the oocyst stage. That is how that although the total infectivity rate is lower, the oocyst rate is much higher than in Series I. This view is further supported by the low sporozoite rate and the sparseness of sporozoite infestation of the salivary glands observed in Series II.

In the four series discussed above, the value of oocyst infections as an indicator of the transmitting capacity of the species varies considerably. In Series I, the inclusion of oocyst infections in the total infection rate is justifiable as it is very unlikely that the oocysts then observed in the gut would not normally complete their development in much the same manner

as other oocysts had matured and produced sporozoites. In Series III, the infected specimens had only the oocyst stage of the parasite and there was no sporozoite infection of the salivary glands at all. It seems apparent that under the conditions that prevailed at the time, the oocysts did not mature at all or that their development was so greatly suppressed or retarded as to render sporozoite infection ineffective. Under such conditions, it would not be justifiable to take into consideration oocyst infections in judging the transmitting capacity of the species. The same thing holds good in regard to Series II also. The sporozoite infection of the salivary glands observed in two specimens in that series are evidently the remains of an old infection of the glands which was gradually disappearing, the oocysts in that series did not appear to be developing well enough to produce sporozoites in due time. It is thus seen that while in Series I, the oocysts were capable of maturing and producing a sporozoite infection of the salivary glands, those observed in Series II and III were apparently incapable of doing so. As such, the value of oocyst infections as an indicator of the transmitting capacity even of the same species is dependent on the period of observation.

THE RELATION OF THE OOCYST RATE TO THE SPOROZOITE RATE

With reference to Series I discussed above, it was mentioned that the oocysts observed in the gut are potential sources of sporozoite infection of the salivary gland and that most of them would, as a matter of course, mature and liberate sporozoites. Should such be the case the incidence of oocyst infections and of sporozoite infections of any series would vary in relation to the interval between the day of collection and the day of dissection. One would naturally expect that the incidence of oocyst infections would be highest soon after the date of collection and that it would fall rapidly as the interval between the date of collection and the date of dissection becomes longer. The sporozoite infection, on the other hand, would show an increase with such delay.

Such a phenomenon has been observed in the course of observations on Series I. The mosquitoes that comprise that series were collected every day between 28th October and 9th November 1930. The number of specimens obtained for dissection during this period was so large that the accumulated material could not be dissected out and examined immediately. They were, out of necessity, dissected on different dates following the date of collection. The results of these dissections have been classified and tabulated according to the interval that elapsed between the date of collection and the date of dissection. These tabulated results show the variations of the oocyst rate and the sporozoite rate in relation to the interval between the date of collection and the date of dissection. The following table gives the results of these observations.

TABLE I

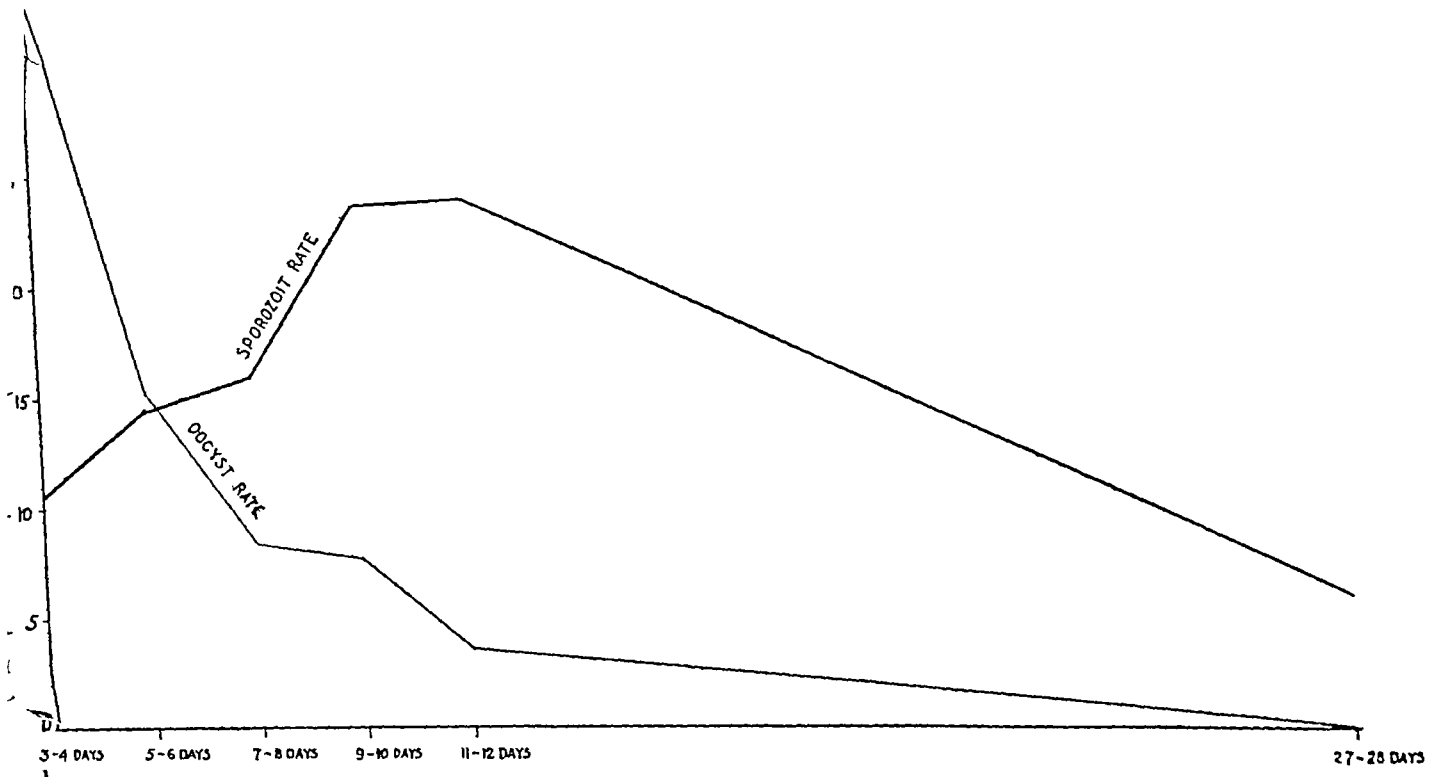
Period between date of collection and date of dissection	MIDGUTS			SALIVARY GLAND			TOTAL INFECTION		
	Number examined	Number with oocysts	Oocyst rate	Number examined	Number with sporozoites	Sporozoite rate	Number examined	Number with oocysts or sporozoites or both	Infection rate
3 to 4 days	21	7	33.3	19	2	10.5	21	7	33.3
5 to 6 days	118	18	15.3	118	17	14.4	119	24	20.3
7 to 8 days	144	12	8.3	145	23	15.8	145	28	19.3
9 to 10 days	392	30	7.6	391	92	23.5	392	101	25.8
11 to 12 days	144	5	3.5	144	34	23.6	144	35	24.3
27 to 28 days	17	0	0.0	17	1	5.8	17	1	5.8

The earliest figures available are of the 3rd to 4th day after the day of collection, no figures are available for less than that period of interval owing to the fact that no dissections were made until the stomach was clear of the blood meal.

These observations show that the incidence of oocyst infections is highest in dissections in which the interval between the day of collection and the day of dissection is shortest and as the interval becomes longer, the incidence of oocyst infections decreases rapidly. On the other hand, the incidence of sporozoite infection is low in dissections carried out during the first few days after collection and it increases rapidly with the delay in the date of dissection. The oocyst rate and the sporozoite rate in these observations seem to behave differently, the former drops with a delay in the date of dissection while the latter shows a rise with the delay. The results are graphically represented in Chart 2. The oocyst rate is highest, namely 33.3 per cent, during the 3rd and 4th day after date of collection and diminishes rapidly as the interval increases. It falls to 15.3 per cent in 5 to 6 days, to 8.3 in 7 to 8 days, to 7.6 in 9 to 10 days and finally to 3.5 per cent in 11 to 12 days. On the other hand, the sporozoite rate increases proportionately with

the decrease of the oocyst rate and the increase in the intervening time between the day of collection and the day of dissection. It is here observed to start with a 10.5 per cent figure on the 3rd to 4th day, it rises to 14 and 16 per cent respectively in the two subsequent groups and finally reaches as high a figure as 23.5 per cent in 9 to 10 days. Thereafter it remains steady for a while and subsequently experiences a slow and gradual decline.

CHART 2



The explanation of these observations is apparent. At the time of capture, a certain intensity of sporozoite infection and of oocyst infection occur among these mosquitoes. With the lapse of time, the oocysts grow, mature and burst, liberating sporozoites. As the mosquitoes in captivity do not get any further infective feeds, no more fresh oocysts can develop in the midgut. Since no fresh oocysts are formed to replace those that mature and burst, the oocyst rate drops in proportion to the maturing of the oocysts, the latter being directly dependent on the interval between

the last infective feed and the date of dissection. Proportionately with the fall of the oocyst rate, the sporozoite rate increases until it reaches a high level about the 9th day after capture. From that point on to the 12th day, the sporozoite rate was observed to maintain the same level, as by that time most of the oocysts had evidently matured, burst and discharged their sporozoites. After this stage, the sporozoite rate declines slowly as seen from the fall from 23.6 per cent on the 11th and 12th day to 5.8 per cent at the end of 28 days. This fall in the sporozoite rate is the result of continued discharge of sporozoites during each occasion on which the mosquito feeds on the raisins kept in the mosquito cages and the consequent emptying out of the glands of their sporozoites. This matter will be referred to again later.

THE OOCYST RATIO AND THE SPOROZOITE RATIO

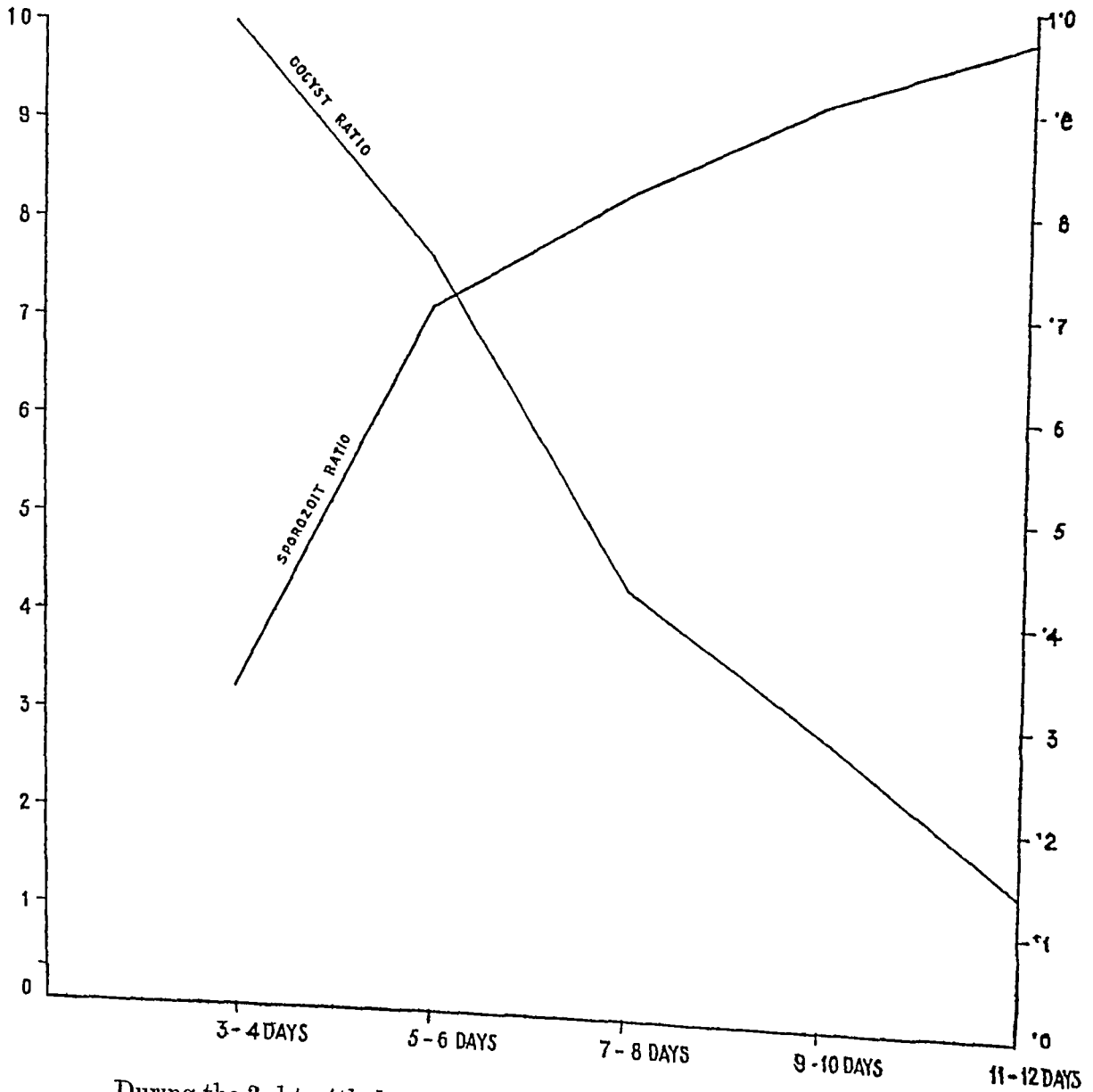
Chart 2 is based on the oocyst rates and the sporozoite rates observed in the series. There is, however, the possibility of error due to chance variations resulting from random sampling. There is no certainty that the degree of infection in the 3 to 4 day group is equal to that of any other group. If, on the other hand, instead of taking the actual percentages, the different ratios of the oocyst rate and the sporozoite rate to the total infectivity rate of each group are taken into consideration, such error due to variations in random sampling would be entirely eliminated. In Table II below, the variations of the two ratios above mentioned are shown in relation to the interval between the day of collection and the day of dissection. The term 'oocyst ratio' is given to the ratio of the oocyst rate to the total infection rate, and by 'sporozoite ratio' is meant the ratio of the sporozoite rate to the total infection rate.

TABLE II

Interval between the day of collection and the day of dissection	Oocyst ratio (oocyst rate divided by infection rate)		Sporozoite ratio (sporozoite rate divided by infection rate)	
3 to 4 days	$\frac{33.3}{33.3}$	1.0	$\frac{10.5}{33.3}$	0.32
5 to 6 days	$\frac{15.3}{20.2}$	0.76	$\frac{14.4}{20.2}$	0.71
7 to 8 days	$\frac{8.3}{19.3}$	0.43	$\frac{15.8}{19.3}$	0.82
9 to 10 days	$\frac{7.6}{25.8}$	0.29	$\frac{23.5}{25.8}$	0.91
11 to 12 days	$\frac{3.5}{24.3}$	0.14	$\frac{23.6}{24.3}$	0.97
27 to 28 days	$\frac{0.0}{5.8}$	0.0	$\frac{5.8}{5.8}$	1.0

These figures show how the oocyst ratio starts high and falls rapidly with the increase in the interval between the date of collection and the date of dissection, it exhibits a negative correlation with the interval period. The sporozoite ratio, on the other hand, starts low and rises as the interval period gets longer, it shows a positive correlation with the dissection interval period. These results are graphically illustrated in Chart 3.

CHART 3



During the 3rd to 4th day after collection, the oocyst ratio is at 10, from this high point, the oocyst ratio gets less and less, finally reaching nearly zero as a result

of the maturing of the oocysts and their collapse after they had discharged the sporozoites. The sporozoite ratio, on the other hand, starts with a comparatively low figure, namely 0.32, during the 3rd and 4th day after collection and rises rapidly with the increase in the interval period. It finally reaches 1.0 when all the infected specimens have acquired a sporozoite infection of the salivary glands.

The results discussed above show that the relative incidence of sporozoite and oocyst infections in any series of dissections of a carrier species depends largely on the interval that elapses between the day of collection of the specimens and the day of dissection. This has been demonstrated above by a consideration of the actual sporozoite rates and the oocyst rates, as also by a consideration of the sporozoite ratios and the oocyst ratios, whereby the chances of error in random sampling are entirely eliminated. If the observations are made within a few days after the date of collection, the oocyst infection tends to be the highest of the series and the sporozoite infection the lowest. If the day of observation is delayed, the oocyst infection decreases and the sporozoite infection increases in proportion to the length of the interval between the day of collection and the day of dissection.

LOSS OF SPOROZOITE INFECTION

In discussing the variations of the sporozoite rate (Table I), it was observed that the sporozoite rate fell from 23.6 per cent during the 11th to 12th day after collection to 5.8 per cent at the end of 28 days (Chart 2). This is a definite fall in the incidence of the infection and it was mentioned that it may be due to the continued emptying out of the sporozoites from the salivary glands as a result of the mosquitoes feeding on the raisins kept in the mosquito cages. It was also observed that the infestation of the salivary glands with sporozoites at the end of 28 days was decidedly poor, which indicates that the infection had been getting to be less and less. This diminution in the sporozoite infection rate as well as in the intensity of infestation of the salivary glands is the result of the discharge of sporozoites during every time the mosquitoes fed on the raisins kept in the mosquito cages. As a result of the maturing of the oocysts present in the gut, the maximum sporozoite infection and the highest infestation occurred during the 9th to 12th day after collection. When all the oocysts had matured, there is no further supply of sporozoites to infect the salivary glands and from this stage onwards there is a constant discharge of sporozoites during every occasion when the mosquito feeds on the raisins. As no more sporozoites come up to replace the ones lost during the process of feeding, the sporozoite infestation gets lower gradually with the result that the sporozoite infection rate also depreciates in course of time. The longer the lapse of time after the optimum infection period, the greater is the reduction in the infection rate.

The same phenomenon would happen even under natural conditions. In the above observations, the mosquitoes, which were in captivity, were deprived of any

further infective feeds after the time of capture. In nature the same effect can be produced by the onset of an adverse season with the result that although the mosquito may get an infective feed there is no more formation of oöcysts and the oöcysts already in the gut are suppressed in the course of development. The result of such an event would be that the sporozoite infection which exists in those mosquitoes at the time of the onset of the adverse season would tend to be gradually discharged with every act of feeding. The result of this process would be that in course of time the sporozoite infestation would diminish rapidly and the sporozoite rate would fall. The results of the observations on Series II discussed in an earlier part of this article (page 528 *ante*) in which the sporozoite infestation was very sparse and the sporozoite infection rate suddenly fell from 20.3 per cent in Series I to 3.7 per cent in Series II during the course of a month, illustrate this phenomenon of such natural loss of infection.

LIFE-HISTORY AND MORPHOLOGY OF *TRYPANOSOMA*
PHLEBOTOMI (MACKIE, 1914).

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[Received for publication, May 9, 1931]

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I GENERAL OBSERVATIONS

CONSIDERING the large size of the genus *Trypanosoma*, if all the species attributed to this genus are to be considered valid, it is surprising that the complete life-histories of only a comparatively few species are known if, indeed, the word complete is not an exaggeration in this connection. The importance of the genus, both on account of its world-wide occurrence and its widespread distribution as parasites in the animal kingdom, for its hosts include members of such widely separated phyla as the *Arthropoda* and *Vertebrata*, warrants the description of as many complete life-histories as possible from hosts at least of different families. Only when a sufficient number of these are known will our knowledge of the genus *Trypanosoma* be adequate for the separation of species within it based on precisely ascertained data on morphology and bionomics, the former, in our present state

(541)

of knowledge, being still the most important character in differentiating protozoan species

Bearing these points in mind, when we encountered, during the course of work on kala-azar, a trypanosome parasite in lizards of the genus *Hemidactylus*, family *Geckonidae*, and in sandflies of the genus *Phlebotomus* the opportunity given us to work out the life-history of this trypanosome was gladly seized, not only on account of its intrinsic interest but on account of the hope that it would throw some light on the major study of kala-azar on which we were engaged. The account which follows represents the result of our study of this interesting parasite and, it is hoped may serve as an example of a life-history for comparison with the life-histories, still to be worked out, of other members of the genus *Trypanosoma*

II SYNONYMY

The parasite which is the subject of this study was first described by Mackie (1914) under the name of *Herpetomonas phlebotomi* which was found parasitic in the sandfly *Phlebotomus minutus*

Mackie, Gupta and Swaminath (1922) later named what was probably the same parasite, now recovered from the gecko lizard *Hemidactylus frenatus*, Dum and Bibr, *Trypanosoma hemidactyli*. In the same paper they described a crithidial parasite of the sandfly, *Phlebotomus minutus*, and named it *Crithidia phlebotomi*, unaware that they were dealing with different stages in the life-history of one parasite. In 1924, Shortt, in recounting the parasites found in sandflies of the genus *Phlebotomus*, remarked that the trypanosome of the lizard and the crithidia of the sandfly, both of which he also had encountered, were probably one and the same parasite, a possibility which had also been envisaged by the writers previously referred to

In 1925, Shortt, from a study of Mackie's twelve-year old original preparations, which had faded considerably, described the parasite as *Bodo phlebotomi*, being misled by the fact that there were many dividing forms possessing two flagella although the nucleus did not appear to have divided or to be in process of doing so

The study of an abundance of material, both lizards and sandflies, has now enabled us to unravel the tangle of names and we are on firm ground when we pronounce that the *Herpetomonas*, *Crithidia* and *Bodo* of the sandfly and the trypanosome of the lizard are one and the same parasite, the name of which therefore according to the rules of Zoological nomenclature must be *Trypanosoma phlebotomi* (Mackie, 1914). Previous work on *Trypanosoma phlebotomi* left one in some doubt as to the precise species of both the reptilian and insect hosts of this parasite and this preliminary point should first be cleared up

The reptilian host has now been definitely identified for us by Mr Parker of the British Museum as *Hemidactylus frenatus*, Dum and Bibr, and the insect host by Lieut-Col J A Sinton, v c i m s as *Phlebotomus babu* var *shortti*, Adler and Theodor

III LIFE-HISTORY

In treating of the life-history a short description of the trypanosome as found in the lizard will first be given and this will be followed by descriptions of its stages of development in the order in which they occur in the sandfly when the latter ingests the parasite in its blood meal off the lizard. The completion of the life-history by the retransference of the parasite to the lizard after the completion of its development in the sandfly will be finally considered.

A *Trypanosoma phlebotomi* in *Hemidactylus fienatus*

Hemidactylus fienatus is a wall gecko very common in Assam. It may be found throughout the year but is rare in the coldest months when its insect food is at a minimum.

Technique —To obtain blood from the gecko for examination for trypanosomes is not easy unless the correct technique is followed. This depends on an exact knowledge of the position of some convenient large blood vessel closely underlying the skin. The simplest and surest procedure is as follows. The lizard is grasped by the thumb and forefinger of the left hand, the thumb being applied to the under and the forefinger to the upper surface of the head. With a sharp flat surgical needle a quick 'jab' is made in the neck region about $\frac{1}{2}$ mm. behind the ear hole. If the underlying vessel is successfully pricked there is an immediate formation of a drop of blood at the site of the puncture. It is picked up on the edge of a glass slide and a smear made on a second slide, or the drop may be transferred as such to another slide and covered with a slip for observation in the fresh state. By the latter procedure an immediate diagnosis of the presence or absence of trypanosomes may be made. To prevent clotting of the blood the drop may be mixed on the slide with a small drop of citrated saline solution (NaCl 0.8 per cent, Sod Cit 1.5 per cent) and, in any case, the preparation should be sealed with melted vaseline.

As the morphology of the parasites is to be treated in later sections and we are now dealing with the life-history, the appearances seen in a fresh preparation only will now be considered. The presence of parasites in a preparation is at once evidenced by a movement among the blood corpuscles. This is seen to be caused by a large undulating parasite about one and a half times the long diameter of the red blood cells. The parasite is somewhat elongated but, at times, may be ovoid or even nearly circular. It is hyaline in appearance and its movements are seen to be produced by the rhythmical undulations of a membrane which extends the whole length of the parasite. The movement is not limited to the membrane for the waves of the latter are communicated to the body proper of the parasite which moves in rhythm with it. The undulating movement described may go on without the parasite appreciably changing position or there may be, in addition, a movement

of translation, but this is never rapid in character nor large in amount and is not obviously co-ordinated to produce continued progression in any particular direction. The forms seen in the peripheral blood are monomorphic.

The percentage of infected lizards we found to be 74, based on the results of one examination only of each lizard. These examinations were made throughout the year except in the months of November and December and the results did not reveal any variation in the percentage of infections sufficient to indicate any definite seasonal variation. From this we deduce the probability that infection once acquired lasts for a long time if not, indeed, for the life of the lizard. It appears to be quite innocuous to the host.

B *Trypanosoma phlebotomi* in *Phlebotomus babu* var *shortti*

(a) Ingestion of the parasite by the fly

If an infected lizard be confined in a small muslin cage within a frame with a glass top and laboratory bred (and hence clean*) *Phlebotomus babu* var *shortti* be released therein the latter will soon begin to feed upon the lizard. Any situation on the latter may be the seat of attack and the lizard appears to be quite unconcerned, making no effort to get rid of the flies. The latter take a considerable time to make their blood meal, ten to twenty minutes or longer elapsing before the process is complete. The most active movements of the lizard do not appear to disturb the flies in the process of feeding. When replete they are comparatively inactive and tend to seek any available shelter to digest their meal in safety.

(b) Stages in development up to twenty-four hours after ingestion by the fly

As we have never found the lizards heavily infected there is great difficulty in identifying the one or two individuals ingested by the fly among the relatively great mass of blood. This difficulty is accentuated by the fact that the trypanosomes seem to lose their motility very rapidly in the fly and, in addition, the blood becomes coagulated or at least assumes a condition which renders it difficult to disintegrate it in a search for the contained trypanosomes. As a consequence of these difficulties the earliest stages observed by us in fresh preparations were at about three to five hours after ingestion (Plate XXXIV, fig 17). The blood at this time is still nearly fresh in appearance although more or less consolidated. The trypanosomes ingested at the meal have lost their characteristic shape and are now represented by ovoid or nearly spherical bodies measuring about 10μ in diameter. There is now no trace of any undulating membrane and consequently

* The method of breeding *Phlebotomus* flies of the 'minutus' group is exactly similar to that described by Shortt, Barraud and Swaminath for *P. argentipes* in *Ind Jour Med Res*, XIII, p 943

no power of movement. In these bodies the cytoplasm is very markedly vacuolated and extremely refractile due to highly refringent granules occupying and filling the spaces between the vacuoles. Around the body is a distinct periplastic membrane.

From this stage onwards multiplication of the parasites proceeds at a great rate. One such body kept under observation had, at the end of an hour, already divided into four individuals (Plate XXXIV, fig. 16).

At this stage there was present, also, an entirely new feature. Surrounding the four daughter bodies was a definite hyaline cyst wall which seemed to represent the original periplastic covering of the previously undivided body. The four daughter individuals contained within the cyst were ovoid in shape and measured about 4.5μ in their longest diameter. Their contents were masses of highly refringent granules lying within a hyaline matrix. From the four-individual stage there follows a progressive increase in size of the bounding cyst wall *pari passu* with an increase by division in the number of contained bodies. After the lapse of about twenty-four hours from the ingestion of the parasites by the fly a characteristic stage has been reached which will now be described as it is seen in fresh preparations and in stained sections of the fly.

In fresh preparations

At this stage the midgut of the fly dissects out very easily, either posteriorly or anteriorly, the blood meal being, apparently, a more or less solid clot. If the preparation be covered with a slip and flattened out by pressure the gut wall will give way and the contents can then be more easily examined while the disintegration of the mass is not sufficient to destroy the general relation of parts, so that anterior and posterior parts of the gut, and the contents corresponding, can still be correlated. The blood meal is represented by a reddish brown granular mass which disintegrates in lumps, indicating that it is solid. In any situation in this mass, but most usually towards its anterior part, one or more clear spheres measuring about 38μ may be seen. These should be manipulated away from the blood mass for more exact examination. When this is done each sphere is seen to be composed of a very definite spherical cyst membrane enclosing contents which completely fill it. The contents consist of a large number, 40 to 60, of very highly refractile bodies lying in a granular matrix (Plate XXXIV, fig. 15). Each of these bodies represents a trypanosome and many of them are seen to be in pairs, representing the rapid multiplication which is going on. The individuals are quite motionless since, at this stage, there is no trace of any flagellum. So far as we are aware there is no previous description of the formation of a definite cyst within which the earlier stages of trypanosomes develop.

The only description we know of having any resemblance to the forms to be described by us is that of the intracellular phase in the development of *T. lewisi*

of the rat as given by Minchin and Thompson (1915) There are, however, fundamental differences of which the most important are that in the case of *T. lewisi* the spheres are intracellular and the individual trypanosomes are flagellated, whereas in *T. phlebotomi* the spheres are free in the midgut yet contained in definite cysts and the individual trypanosomes contained are definitely aflagellate That the containing wall we have described really is a resistant cyst wall we were able definitely to demonstrate as follows

To a cyst at the stage we have described or to one at a somewhat later stage of development (36 hours), an intra-vitam stain, such as dahlia, dissolved in normal saline solution is introduced under a coverslip If the stain is allowed to act for some time it will be seen that the developing trypanosomes in the cyst remain entirely unaffected while their surroundings are stained in greater or lesser degree If, now, the cyst be ruptured by pressure upon the coverslip it will be found that the extruded trypanosomes immediately take up the colour of the dahlia proving that they were previously prevented from doing so by a protecting cyst membrane The latter may now be seen as a crumpled and deflated bag perhaps still containing a certain number of trypanosomes Mention of the cyst wall will be made in connection with later stages of development and further consideration of it may therefore be deferred

In sections stained with non hæmatoxylin

The distribution of the developing trypanosomes in their cystic envelopes with relation to the distended midgut has been considered in the last section and, therefore, the appearances seen in a stained section may be described without further preamble

(1) *Under a low power* —(Plate XXXV, fig 18) Embedded among the debris of the blood meal, in which the nuclei of the red cells may still be distinguished, are seen one or more distinctively differentiated clear, circular or ovoid areas measuring about 38μ in diameter If one of these be examined it is seen to consist of a bounding wall, the space within which is packed closely with ovoid clear bodies each of which shows a distinct darkly staining dot

(2) *Under a $\frac{1}{1-}$ oil immersion lens* —(Plate XXXI, fig 1) Under the increased magnification it is at once apparent that the interior of the cyst is divided up into compartments or meshes by a well defined network The lines composing the boundaries of the meshes, and which are, of course, sections of the walls bounding the compartments, are much stouter towards the centre of the cyst and taper towards the bounding wall of the latter Within each compartment formed by the network lie one or more rounded or ovoid bodies each of which represents a developing trypanosome Each of these exhibits a larger, more lightly-staining nucleus and a smaller more densely-staining parabasal body about one-third or one-fourth the diameter of the nucleus No trace of a flagellum is present

(c) Stage of development reached at the end of thirty-six to forty hours after ingestion by the fly

In fresh preparations

The midgut is easily extracted by the posterior route. The contents are a more or less consolidated mass consisting of partially-digested blood, usually situated anteriorly, and of enormous masses of developing trypanosomes. These masses, which are roughly spherical, are each developed from one of the cysts described in the last section and are seen to be subdivided into smaller contained masses which may be seen bulging on the surface of the larger body. There may be as many of these larger masses as there were cysts at an earlier stage and each is surrounded by a definite cyst membrane which shows considerable resistance to rupture and which impedes the entrance of an intra-vitam stain. On release of the contents of the cyst the latter remains as a torn envelope. The developing trypanosomes inside the large masses are seen as ovoid highly refractile bodies, singly or in small groups and, apparently, quite motionless. If, however, the cyst be ruptured, it will be found that among the great majority of motionless refractile bodies a few may be seen here and there which are actively motile due to the formation of flagella. These flagellate forms are few in number at this stage and appear to have little power of translatory movement although their flagella wave vigorously. If an attempt be made by means of dissecting needles to break up one of the cysts, it will be found that considerable difficulty is experienced in liberating the individual parasites. The natural presumption is that they must be held together by some secretion or by some containing structures. In the description of stained sections it will be seen that the latter is probably the true explanation.

In sections stained with iron hæmatoxylin

(1) *Under a low power* —(Plate XXXV, figs 19 and 20) In a median saggital section of the fly it will be seen that a large, if not the main, portion of the midgut is now occupied by huge developing cysts, each representative of one of the small original cysts described at the twenty-four-hour stage, while the intervening spaces are occupied by still undigested blood debris. These cysts are no longer strictly circular or ovoid, being somewhat irregular in outline owing to mutual compression against each other or against the anatomical structures of the fly. The individual cystic masses may measure about 78μ in diameter and have a very characteristic structure which will now be described.

(2) *Under a $\frac{1}{2}$ oil immersion lens* —(Plate XXXI, fig 2) Surrounding each cyst is a very definite cyst wall more or less hyaline in structure and conforming in outline to the shape of the space occupied by it, while at the same time tending to maintain its regular circular or ovoid form. This cyst wall is seen to be entirely distinct from the mere condensation of the gut contents around its periphery and is

well seen in places where shrinkage in fixation has caused the gut contents to recede from it

The entire interior of the cyst is divided up into a large number of spaces irregular in size and shape, bounded by a network of fine hyaline lines representing the cut walls of irregularly shaped compartments. At the periphery of the cyst these lines connect with the outer cyst wall. These compartments are seen to be filled, sometimes somewhat loosely, sometimes closely packed, with large numbers of ovoid developing trypanosomes. The vast majority of these are aflagellate but a very few, and these tending to be more elongate, are provided with comparatively long flagella. Each individual has a clearly marked nucleus and parabasal and many, if not the majority, are obviously at some stage in division. The size of the individuals is considerably less than those at the twenty-four-hour stage and this fact is probably the result of rapid and continuous multiplication.

(d) Stage of development reached at the end of seventy-two hours after ingestion by the fly

In fresh preparations

The general appearance of a dissection made at this stage is not markedly different from that seen at the thirty-six-hour stage when considered as a whole. A more close examination, however, of the developing cysts will show that a definite advance in development, indicating a profound morphological change in the individual parasites, has been reached. The general reticular character of the interior of the large cysts is retained and these cysts may now, for a reason to be presently explained, be termed the *primary* cysts. Within the primary cysts much smaller groups of parasites are seen, indeed these groups now form the main constituents of the primary cysts, in contradistinction to the smaller groups or single or dividing aflagellate trypanosomes described in the last stage. If one of the primary cysts be ruptured and the groups described be extruded, a striking transformation will be seen to have taken place. Each of the smaller groups present in the primary cyst will now be seen to be itself a cyst containing trypanosomes and may therefore be referred to as a *secondary* cyst.

Each secondary cyst when set free is seen to be oval in outline and measures about 9.5μ in its longest diameter. It consists of a very delicate cyst wall enclosing a space completely filled with a writhing mass of flagellates. The motion within the secondary cyst is one of intense seething motility giving the impression that the elongate flagellates are continuously sliding or gliding over one another but, withal, so tightly packed that the individuals are inextricably mingled together. If one of the secondary cysts be kept under observation for some time it will be seen to disrupt and long actively motile flagellates detach themselves and swim rapidly away. Such motile forms may be seen in large numbers throughout the preparation and they are active enough to swim rapidly out of the field of view.

In sections stained with iron haematoxylin

(1) *Under a low power* —(Plate XXXV, fig 21) The appearance under a low power does not vary markedly from that seen at the thirty-six to forty-hour stage. The same reticular structure is markedly in evidence, the interspaces being filled with what appear to be somewhat larger groups of parasites than in the previous stage.

(2) *Under a $\frac{1}{12}$ oil immersion lens* —(Plate XXXII, fig 3) The first glance under a high power reveals that a striking change has occurred in the parasites. The reticular structure, also, appears in some cases to be further modified. This modification takes the form of a still greater subdivision of the chambers described in the last stage. This is evidenced by the persistence of the older and coarser subdivisions of the cyst while the spaces formed by them have undergone a further subdivision into still smaller spaces. The smaller chambers so formed contain each one group and, we believe, one group only of parasites. These groups are seen to be now multinucleate and vary in shape from sub-ovoid to elongate. Each of these multinucleate groups represents one of the secondary cysts described in the fresh preparations examined at this stage. The nuclei stand out with great distinctness retaining the stain tenaciously. Within the secondary cysts the individual parasites are arranged on the whole longitudinally in the long axis of each cyst. Although the majority of these parasites have probably by now acquired flagella yet so tightly are they packed inside the secondary cysts that these are not demonstrable except in those individuals which have escaped from disrupted cysts.

(c) Stage of development reached at the end of one hundred and thirteen hours after ingestion by the fly

In fresh preparations

On opening the midgut enormous masses of parasites emerge. Some of these masses appear to be very similar to those described in the last stage, i.e., large primary cysts enclosing large numbers of secondary cysts. In addition to these enormous numbers of actively swimming free flagellates, very elongate in shape, are present which give the whole field an appearance of intense activity. These free forms may be found invading the hindgut of the fly and considerable numbers may even have reached the rectum where they can be seen very actively motile around the rectal papillæ.

From the description given of the stage at seventy-two hours one would infer that, as the secondary cysts developed and discharged their flagellates, so the latter free swimming forms would increase in numbers at the expense of the less developed stages. This, however, does not appear to be the case and our interpretation of the observed facts is as follows. By the fourth or fifth day there appears to be established a certain relationship of the parasitic infection to the alimentary canal of the fly which persists for the life of the latter, even when it feeds a second time.

The conditions of this relationship seem to be that the midgut of the fly appears to have been constituted a manufactory for the continual production of flagellated forms which, on being set free in the midgut, tend continuously to join a stream of migration in the direction of the rectal ampulla. In other words, the formation of secondary cysts is repeated indefinitely in the position established by the infection in the midgut and continues as the source of supply of flagellated forms which, owing to their power of free swimming are enabled to make their way toward the part from which the infection is presumably to be transmitted to the vertebrate host. As to the source from which the continuous supply of secondary cysts is derived we are uncertain. It may be that while a majority of the flagellates freed by the rupture of the secondary cysts is destined to migrate posteriorly towards the rectum, a minority is destined immediately to commence division and to form a new generation of secondary cysts.

In sections stained with non hæmatoxylin

(1) *Under a low power*—In the midgut the condition seen is almost the same as that described for the last stage and need not be further enlarged upon. In the hindgut there is as yet little to be seen but in the rectum, occupying the spaces in the rectal ampulla around the papillæ, considerable numbers of flagellates may be made out although their details are not visible (Plate XXXV, fig. 22)

(2) *Under a $\frac{1}{2}$ oil immersion lens*—

(a) *The midgut*—Here the condition seen is very similar to that described for the last stage. There is the same great development of secondary cysts but there appears to be some difference in their relationship to the containing chambers. Whereas at the seventy-two-hour stage each chamber contained but one secondary cyst, a certain number now appear to contain two or more. Whether this is actually the case or is more apparent than real, and due to the breaking down of adjacent chamber walls by the escaping flagellates we are unable to say. Free elongated flagellates are to be seen but in small numbers compared to the appearances seen in a fresh preparation. This is probably due to the fact that in the latter case large numbers are set free by trauma from mature secondary cysts, a complication which is absent from preparations in sections where the objects remain *in situ*.

(b) *The hindgut and rectum*—(Plate XXXII, fig. 4) The hindgut is very rarely satisfactorily seen in sections. This is due to the development of the ova after the fly feeds. The ovaries then come to occupy most of the abdomen and, as the more fully developed ova never fix satisfactorily, they cut indifferently and so prevent the hindgut, which passes between the ovaries, from giving a good preparation. At best, parts of it may be seen here and there among the masses of ova and in them a few elongate flagellates may be distinguished.

The rectum, as a rule, gives a good preparation in sections. Into the rectal ampulla may be seen projecting the two rectal papillæ. In the space between these

are large numbers of elongate flagellates with long flagella. So far as can be ascertained all of these flagellates are cithridial in type and the true trypanosome type is conspicuous by its absence.

The stage which we have now reached in the life-history of the trypanosome parasite, viz., the first invasion of the rectum of the fly by free swimming cithridial forms, marks also a crisis in the life of the host. The eggs are mature and, until successful oviposition takes place, the fly is unable to feed a second time and will die. Provided the eggs are successfully deposited the fly is ready for its second feed on a lizard and, on completion of this, the stage is set for the final act in the life-history of its trypanosome parasite.

(f) Final stage of development reached four or five days after the second feed of the fly (eighth to ninth day after ingestion by the fly)

In fresh preparations

On opening the midgut it is seen that the intensity of infection dwarfs anything previously seen. All the stages in the individual trypanosomes already described, except the blood forms, are to be encountered, but the main impression conveyed to the eye is an intense seething activity caused by the innumerable elongate free forms darting about in every direction and transferring a part of their own activity to objects not in themselves motile. At this stage this intense activity is not limited to the midgut but reaches every part penetrable by the free swimming forms so that the infection also extends up the malpighian tubules to a considerable distance. The activity of the free motile forms in the midgut to some extent masks the presence of the earlier stages of development but on examination it is evident that these are probably present in greater number even than the more striking free forms. The hindgut and rectum are similarly tightly packed with parasites, the potential space normally present between and around the rectal papillae being entirely occupied by the parasitic mass. So tightly compressed may the mass distending the hindgut and rectum be that no movement of the parasites may be visible. If, in such a preparation, the gut or rectum, which is filled with an apparently inert mass, be ruptured, an indescribable activity is released indicative of the degree to which the compression precluding movement was operative, in spite of the fact that this movement is produced almost entirely by long flagellated forms which have been compressed into inactivity by the still greater mass of short 'metacyclic' forms to be seen in fixed and stained preparations and which will be described in a later section.

In sections stained with iron hæmatoxylin

(1) *Under a low power* —(Plate XXXV, figs 23 and 24)

The entire midgut is seen to be occupied by a massive parasitic infection. The parasitic mass as a whole appears to be surrounded by a thick bounding

wall applied against the epithelium of the midgut and of about the same thickness as the latter. Within this bounding membrane, which may represent the cyst wall of a primary cyst, the parasites are arranged in immense multinucleated masses which may measure as much as 31μ in diameter. The reticular structure which has persisted throughout the different stages of development is still retained, the reticulæ bounding the large parasitic masses and penetrating them further to subdivide them. The hindgut, when visible, is packed with parasites so thickly that the latter present a uniform multinucleated filling distending the gut. The rectum, in the space between and around the rectal papillæ, is completely occupied and greatly distended by a continuation of this parasitic filling.

(2) *Under a $\frac{1}{2}$ -oil immersion lens* —

(a) *The midgut* — The appearances seen under the high power differ considerably from those apparent in the earlier stages of development. The immense development in numbers which the secondary cysts have by now undergone has led to their compression into very large masses each of which probably originally consisted of many secondary cysts. In some cases the cysts contributing to the formation of the large masses can still be differentiated. The trabecular structure is still present and the presence of these trabeculæ dividing up the parasitic growth into greater and smaller masses, themselves further subdivided by finer trabeculæ, gives to the whole structure a most striking superficial resemblance to the formation of a secreting gland such as, for instance, the pancreas. The majority of the parasites constituting the larger and smaller masses appear to be elongate forms and the whole parasitic activity in the midgut is analogous, as already stated above, to a manufactory for the production of these forms on a large scale to keep up the stream of migration towards the hindgut and rectum.

(b) *The hindgut and rectum* — (Plate XXXIII, fig. 5) In these situations, and more especially in the rectum, the anatomical parts are enormously bloated with their parasitic contents. These, unlike those of the midgut, tend increasingly as one proceeds to examine in a direction posteriorly from the midgut towards the rectum, to be composed of shorter and shorter forms with shorter and shorter flagella until most of those composing the mass in the rectum are short stumpy forms with only rudimentary flagella, when these are present at all, presumably the so-called 'metacyclic' forms. The state of compression, and this applies equally to the midgut, is too great for the flagella of the parasites to be individually distinguishable.

(g) *Retransference of the infection to the lizard Hemidactylus frenatus*

Owing to the posterior direction which the parasitic development pursues, with the eventual production of large numbers of 'metacyclic' forms in the rectum, the presumption must be that the transference of the parasite to its vertebrate

host, a process necessary to its survival, must occur posteriorly if the insect host takes any active part in the process. The absence of any parasites in the anterior parts of the alimentary canal of the fly precludes infection by the bite of the latter, but this still leaves two possible modes of transference of infection to the vertebrate host to be considered. These are —

(1) *The subcutaneous route*

(2) *The oral route*

The subcutaneous route

The sandfly, as we have stated, takes a considerable time over its blood meal, and in the course of it ejects several drops of a fluid from the cloaca. Whatever be the origin of this fluid, whether from the rectum or from the malpighian tubes, it must necessarily contain flagellates in the case of an infection such as that described, and there is no difficulty in postulating the possible infection of the wound made by the proboscis of the fly in feeding with these flagellates.

The oral route

(a) *Ingestion of rectal contents of the fly by the lizard* — The fly may take its blood meal from any part of the lizard except its ventral surface. The region of the head and neck is quite frequently selected and the transference of the flagellates extruded from the cloaca of the fly in the course of feeding to the mouth of the lizard would present no great difficulties.

(b) *The ingestion of the whole fly by the lizard* — We have never in nature, actually seen a gecko capture a sandfly (*Phlebotomus*), but there seems no reason why it should enjoy any particular immunity and, indeed, we have frequently found that on liberating a known number of *Phlebotomus babu* var *shortti* into a muslin cage containing *Hemidactylus frenatus* in order to infect the flies, we have been unable to recover the number of flies liberated in the cage. The presumption is that some of these flies must have been eaten by the lizard, most probably after they themselves had made their own meal, since in the gorged state they would be larger and more visible.

Whatever the various possible routes for infection may be, we considered the oral to be the most probable and to verify this carried out the necessary experiments. Very young lizards newly hatched from eggs were utilized. These were almost certainly uninfected but, to make sure, were examined for trypanosomes and found uninfected.

They were fed, at intervals of a few days, on *P. babu* var *shortti* which had been infected by feeding on infected lizards and then refeeding after oviposition. This resulted in the infection in the flies being eight or nine days old so that the infective stage in the rectum was well established. The blood of the lizards was examined

after two or three weeks for the presence of trypanosomes. The details of two successful experiments are as follows —

Animal	Number of times fed on infected flies	Total number of flies given	Days after first feed when blood was examined	Result
<i>H. frenatus</i> 1	3	5	25	Lizard infected with <i>T. phlebotomi</i>
<i>H. frenatus</i> 2	4	9	17	Lizard infected with <i>T. phlebotomi</i>

It is probable that the infection in the lizards could have been found at an earlier period, but we wished to give the infection every chance to establish itself before making an examination. The reason for this was that the newly hatched lizards were minute creatures not much more than an inch long and did not always survive the extraction of blood. These experiments seem to us to prove beyond reasonable doubt that the method of infection in lizards is normally by the oral route and that the sandfly *P. babu* var. *shorti* is the vector of the trypanosomes. A possible fallacy might lie in the fact that the lizards could have got the infection from some of the muscid flies used to feed them upon during the incubation period of the infection, but we do not think this need be seriously considered. Moreover, other lizards kept under similar conditions, and fed in the same way on muscid flies only did not acquire any infection.

IV MORPHOLOGY

A *The adult trypanosome* (Plate XXXIV, fig 6)

1 *Zoological position* —The general morphology of the parasite places it in the group of the trypanosomes characteristic of cold-blooded animals.

2 *Measurements* —The variations in size of individuals seen in the peripheral blood are not very great and the major variations in lengths and breadths are apparently due chiefly to fortuitous alterations brought about by the relationships of the parasites to the surrounding red cells. The measurements of the lengths and breadths of 15 wet fixed and mounted individuals taken at random were 29.4 μ long and 14.2 μ broad with extremes of 40.5 μ and 24.75 μ long and 18 μ and 11.5 μ broad.

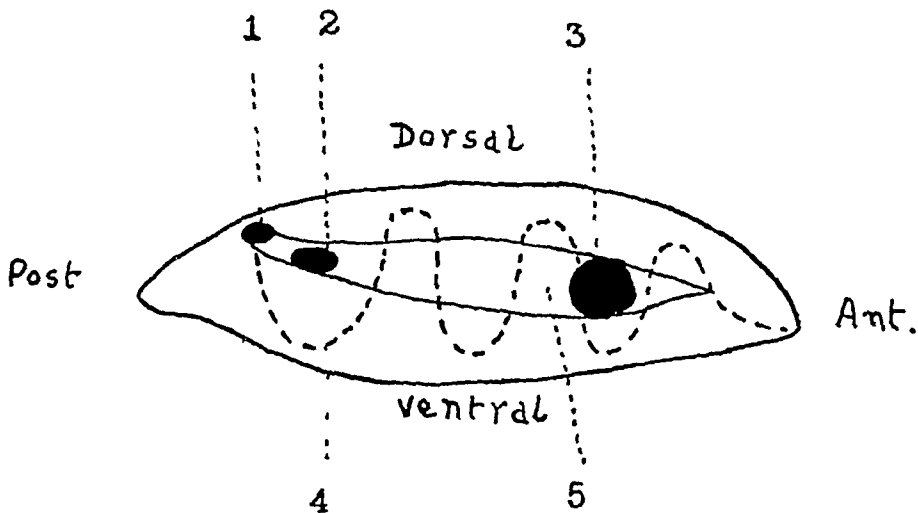
3 *Orientation* —The orientation of the different parts of the parasite with regard to its surface and to its extremities, and their relationships to one another, are fixed and definite when the parasite has its natural shape. Even when this is distorted by outside agencies the distortion seems to affect only the periplast and

the cytoplasm, the internal structures, such as the nucleus, parabasal and axostyle retaining their mutual relationships, a fact which argues a certain rigidity of structure in the interior of the parasite

There would seem to be no reason for considering any particular surfaces of the parasite as dorsal and ventral but, for purposes of description, we may arbitrarily consider as dorsal that surface of the parasite from which the origin of the undulating membrane arises. For the same reason we may call that the anterior end which is farthest from the origin of the same organelle since this would be the end which would lead in progression in the case of more active free swimming trypanosomes. Accepting this orientation of the parasite as a whole the undulating membrane commences on the dorsal surface, passes somewhat diagonally to one of the lateral surfaces and is attached along this surface as far as the anterior extremity

The nucleus occupies a position in the interior somewhat anterior to the central transverse plane. The X-granule (to be later described) is situated in the interior about $\frac{1}{4}$ of the length of the parasite from the posterior extremity and the parabasal about $\frac{1}{2}$ of the length from the same extremity but situated more superficially. The relative positions as seen in a median sagittal section would be as shown in Text-figure 1

The axostylar body is centrally placed in the body of the parasite



Text figure 1

Orientation of structures in adult trypanosome

- 1 Parabasal, 2 X granule, 3 Trophonucleus, 4 Undulating membrane,
5 Axostylar body

4 *Shape and consistency*—The general shape of the adult trypanosomes as they occur in the peripheral blood of the lizard is ovoid in optical section. One

extremity, hereafter called posterior, as being at the end of the body from near which the flagellum arises, is bluntly pointed. A cross section through the equator of the parasite would show the body to have considerable bulk and that this is actually the case is evidenced by the considerable difference in focus under a high power of the microscope between the upper and the lower surfaces in wet fixed and mounted preparations. Also, when examined under a high power binocular microscope, the body is obviously thick and voluminous.

This typical conformation is subject to considerable variations according as the body of the parasite is subjected to the pressure of objects in its surroundings. In this way the body may be more elongated when wedged between two rows of blood cells or more rounded when pressed upon on all sides. Irregular shapes may also be assumed showing that the body consists of a jelly-like and highly plastic cytoplasm surrounded by an equally pliable pellicle or periplast.

5 *Cytoplasm* — As seen in wet-fixed iron-haematoxylin preparations the cytoplasm is markedly granular. This granularity is a true indication of the highly refringent granules seen in the cytoplasm in fresh preparations and this property of refringency is not entirely lost even in the fixed and stained preparations.

Towards the posterior end of the body there tends to be a collection of much larger granules or globules with a distinct outer wall and central clear area, the result of being seen in optical section. These large granules may occur in any part of the cytoplasm but any tendency to agglomeration is usually most marked in the region mentioned. On the other hand when concentrations of the finer granules occur, these tend to be towards the anterior extremity, but there is no definite regularity in their distribution.

The cytoplasm in its outer layers is raised into a series of myonemes which appear to pass more or less longitudinally, but with a slight diagonal bias over the anterior and posterior surfaces of the parasite. The diagonal bias appears to be in the opposite direction on the two surfaces so that the two sets of myonemes, if focused separately, appear to cross one another at an acute angle. If the parasite under observation is much flattened, both sets of myonemes may be seen at the same time and then the effect seen is that of a loose meshwork. Apart from the granules already mentioned the cytoplasm is remarkably free from any inclusions. Local rarefactions of the granular structure are sometimes evident giving the appearance of vacuoles, but these are never sufficiently definite to warrant the assumption that they have any structural significance.

6 *Organelle* —

Trophonucleus The trophonucleus is a well-marked body of somewhat irregular shape varying from a regular quadrilateral shape through various modifications to a triangular form. It is practically never spherical and occasionally presents small indentations dividing it into two equal, or greater and

lesser parts This subdivided form of the nucleus is not an indication of commencing division since none of the other accompaniments of a dividing nucleus are ever present

There is a well-marked nuclear membrane enclosing a more or less homogeneous nuclear structure with a remarkable absence of chromatin granules The chromatin would appear to be uniformly distributed throughout the substance of the nucleus and any apparent aggregations are so obscure as hardly to deserve the name of granules No karyosome is visible In preparations stained with Giemsa stain the nucleus stains very faintly and, in some cases, is difficult to distinguish at all—another proof of the small amount of the chromatin present We have never encountered any parasites in the peripheral blood showing any indications of division, and those parasites encountered in the internal organs appear to be identical with those found in the peripheral blood

Two possible interpretations which might be put on this apparent absence of dividing forms, are either that division, leading to multiplication in the vertebrate host, is only a periodic occurrence and that we never examined the blood during one of these periods, or that the sum total of multiplication may occur as soon as infection with crithidial forms takes place and ceases with the attainment of the adult form

X-granule—We have given this name provisionally to the structure here described as we have been unable, so far, to determine either its function or its homology The X-granule is usually a small spherical deeply-staining granule lying near the posterior extremity of the axostylar body about one-quarter the length of the parasite from its posterior extremity and more deeply situated than the parabasal Instead of the typical spherical form it may assume the form of a very short and thick rod placed transversely to the long axis of the parasite This is, however, unusual and may merely be the result of deformation The X-granule has no obvious connections with any other structure In specimens stained with Giemsa stain the X-granule is quite indistinguishable and were this the only stain used its presence would be quite unsuspected We have no suggestions to offer as to the significance or origin of this granule but its constant presence and definite location with respect to other structures leave no doubt that it fulfils some rôle in the internal economy of the parasite

Parabasal—This is a small spherical antero-posteriorly oval granule about the same size as the X-granule, or a little larger, and situated near the posterior extremity of the parasite about one-fifth of the length of the parasite from that extremity It takes an intense stain with iron hæmatoxylin and, unlike the X-granule, also stains intensely with Giemsa stain Surrounding the parabasal there is usually to be seen a halo-like rarefaction of the cytoplasm which may represent a small flagellar vacuole The parabasal has two connected structures in close relationship with it, the undulating membrane and the axostylar body, both

appearing to commence in its immediate vicinity. It has no visible connection with the X-granule.

Flagellar vacuole—This has already been referred to in the preceding section. While usually assuming the form of a narrow halo surrounding the parabasal it sometimes appears to be considerably larger but is then less definitely demarcated from the surrounding cytoplasm and we are not completely satisfied that the appearance in any case really represents a definite structure as is the case in a leptomonad type of flagellate such as *Leishmania*.

Undulating membrane—The outer edge of the undulating membrane appears to commence in a minute blepharoplast situated to one side of the parabasal at a distance about equal to the diameter of the latter. It appears to reach the surface of the body and then turns to take a somewhat diagonal course towards one of the lateral borders of the parasite where it pursues a sinuous course anteriorly along this border to terminate at the extreme anterior end of the body, there being no projection of a free flagellum beyond this point.

Between the outer edge of the undulating membrane and the body of the parasite lies the membrane proper, its line of attachment to the body following generally the line of the outer edge but without its sinuosities so that it takes a shorter course to reach the same termination. The result of this is that the undulating membrane as a whole is thrown into a series of folds numbering four or five, the folding having a general dorsal-ventral arrangement. The part of the outer border which traverses diagonally the body of the parasite to reach the lateral border has very little, if any, membrane attached, being closely applied to the outer surface of the body for most of its length after it has emerged from its intracytoplasmic position at its commencement (axoneme).

The anterior end of the undulating membrane appears to join tangentially the anterior rounded end of the parasite, there being no evidence in fixed and stained preparations that there is any projection of a free flagellum beyond that point.

The membrane proper is hyaline throughout its length being therefore in marked contrast to the granular cytoplasm of the body.

Axoneme—This term is used to denote the very short intracytoplasmic portion of the axial filament which, with its periplastic covering, forms the outer border of the undulating membrane. It is not always easily seen, but, in some iron-haematoxylin preparations, may be made out as a very fine line extending the thick part of the border of the undulating membrane to a point almost, if not quite, in contact with the anterior end of the parabasal.

The blepharoplast—This body, by which is meant a minute granule sometimes to be seen at the commencement of the axoneme, is not always present in preparations stained with iron haematoxylin, at least in visible form. In such cases it seems that the axoneme passes directly into the parabasal, or the separation may be so small as to be beyond the resolving power of the microscope. In dried

preparations stained with Giemsa, however, the blepharoplast can readily be made out, the greater flattening of such preparations causing a larger gap between the blepharoplast and the parabasal

Third granule—In a certain number of parasites there appears, in addition to the structures already described, a third granule placed in a line with the parabasal and X-granules and anterior to the latter, the three granules being equidistant. This granule is usually absent but appears in the same position in a sufficient number of parasites to warrant its mention. It is roughly of the same size as the X-granule and, when present, is definite enough to warrant the assumption that it has some structural significance.

Axostylar body—This is the name we have applied to a structure which does not seem to conform to the description of any of the various organelles described in other species of trypanosome. Mention has been made by various workers on *Trypanosomidae* (Christophers, Shortt and Bariaud, 1927, McCulloch, 1915, Wenyon, 1913) of structures which have been interpreted as homologous to axostyles but none of these descriptions refers to any structure which is of constant occurrence and of so definite a nature as that which in this parasite we have designated the axostylar body.

This body is of an elongated spindle shape, apparently taking origin at or near the parabasal and extending forwards to beyond the junction of the middle and anterior thirds of the body of the parasite.

While the parabasal may be said to be near or at its posterior extremity the X-granule lies on its central line about one-fifth of its length from its posterior end and the trophonucleus is similarly placed about the same distance from its anterior end.

Its shape is very regular, is demarcated by a very clear-cut bounding wall and, while not quite hyaline in appearance, it stands out in marked contrast to the granular cytoplasm. It is seen both in preparations stained by iron hæmatoxylin and by Giemsa.

In general appearance, so far as it extends, and in staining characteristics it would not be distinguished from the axostyle of a form such as *Trichomonas*.

In parasites more or less markedly deformed by outside agencies the axostylar body tends to retain its form showing that it has a definite and, indeed, marked rigidity.

The position of the axostylar body within the parasite is apparently nearly central and the nucleus is therefore closely applied to it. In this connection we may refer to the description by Christophers, Shortt and Bariaud of a somewhat similar but far less definitely marked body in *Leishmania donovani* which, in disrupted parasites, sometimes shows a 'tache' indicating the position where the nucleus had been closely applied to it.

Whether one is justified in considering the organelle under consideration as a rudimentary axostyle we are not sure, but it was only after a full consideration of other possibilities that we were led to consider this as the most reasonable interpretation

B Developmental forms in the insect host

(1) *Twenty-four hours after ingestion* —(Plate XXXI, fig 1) The parasite twenty-four hours after ingestion by its insect host has already divided repeatedly to produce a large number of individuals. These individuals have a general ovoid contour and average 6.7μ long by 3.6μ broad. Each has a distinct periplast, but there is no trace of any flagellum or undulating membrane. The cytoplasm is granular and markedly vacuolated. The nucleus is roughly ovoid and both it and the parabasal stain well with iron hæmatoxylin, the latter especially showing great tenacity in retaining the stain against differentiation with iron alum.

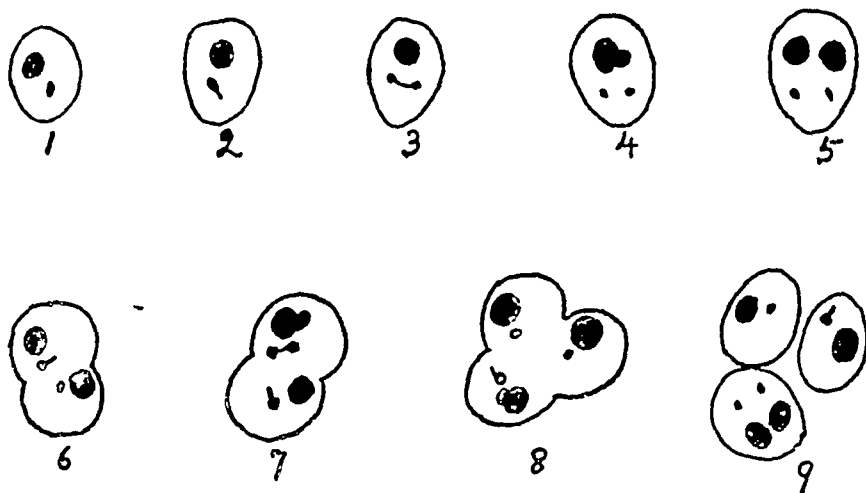
The amount of material at our disposal at the earliest stages of development is very small and we have not, therefore, been able to study in detail the process of division of these early forms. Moreover, division proceeds at such a rapid pace that it is difficult to trace its progress step by step. There is no doubt, also, that division of cytoplasm lags behind that of the nucleus and its associated parabasal with the result that irregularly bulging bodies are formed containing two or more nuclear masses and as many or more parabasals. The bulges are caused by commencing planes of division of the cytoplasm.

As far as can be ascertained the parabasal seems to divide first forming a centrodesmose from which two new daughter parabasals consolidate themselves. This stage may be seen before there is any trace of division in the nucleus. The nucleus then divides in a similar manner, the process being initiated as a small protrusion from one side of the nucleus and the division of nucleus and parabasals may be repeated a second time before the cytoplasm commences to divide. The series of events in division may be represented in diagrammatic form as shown in Text-figure 2.

(2) *Forty hours after ingestion* —(Plate XXXI, fig 2) In describing parasites at this stage there is little to add to descriptions given of those examined twenty-four hours after ingestion. The majority of forms seen are very similar, but there is a tendency towards even greater vacuolation of the cytoplasm. The great majority of the parasites are apparently still aflagellate but somewhat more irregular in shape. This is due, when present, to commencing elongation in the individuals concerned, a first stage on the way to the production of crithidial forms. Multiplication is still very active and there is a greater tendency than ever to the formation of compact agglomerations of parasites the result of rapidly repeated divisions *in situ*. There is some tendency at this stage for the parabasals to assume a distinctly rod-shaped form but we think this may be merely an early sign of division.

in these parasites as described for those at the twenty-four-hour stage. A few of the parasites have already developed flagella and these forms also will be seen to be in active division.

These young flagellates are very short and broad fusiform parasites with well staining cytoplasm (Plate XXXIV, fig 8). In division the parabasal first divides and develops a new flagellum and the latter may be well developed and the parabasals widely separated before the trophonucleus shows any sign of division. This results in the production of forms such as are seen in Plate XXXIV, fig 7.



Text figure 2

Stages in division of the early aflagellate form of *T. phlebotomi* in midgut of *P. minutus* var *shortt* (diagrammatic). Note delayed division of cytoplasm.

(3) *Seventy-two hours after ingestion* — (Plate XXXII, fig 3). At this stage there is at once evident a marked change from conditions present forty hours after ingestion. The same general arrangement of the parasites in groups is evident, but the latter aggregations are larger and contain larger numbers of individuals. The latter, moreover, now include many elongated forms furnished with flagella which, in fact, have assumed a typical crithidial form. As these will be described in the next section no further description of their morphology need be given here.

(4) *One hundred and thirteen hours after ingestion* — At this stage the parasitic activity in the midgut has definitely taken on a double function. One function is the continued multiplication of the ovoid aflagellate forms of parasite which maintain continuously the infection in the midgut. The other function is the development of a large proportion of the aflagellate multiplying forms into long flagellates of typical crithidial form. These crithidia have not the slightest resemblance either to the ovoid aflagellate forms from which they arise and which have already been described or to the adult trypanosomes. They appear to arise in the following way. One of the ovoid aflagellate forms ceases to divide and elongates

slightly becoming pyriform in shape, and developing a short flagellum (Plate XXXIV, fig 8) There is at this stage still no trace of an undulating membrane The pyriform flagellate elongates further and begins to exhibit a rudimentary undulating membrane (Plate XXXIV, figs 9 and 10) From this stage to that of the fully developed mature crithidial forms is only a question of further elongation The fully developed crithidial forms measure on an average 19.3μ in total length of which the flagellum accounts for 10.7μ The form of the body is slightly bulbous posteriorly and tapering very gradually to a point anteriorly so that it is difficult to determine the exact point at which body ends and flagellum begins (Plate XXXIV, figs 11 and 12)

Besides these large forms much smaller individuals are to be seen and there is every gradation between the extremes (Plate XXXIV, fig 13)

Cytoplasm—This is markedly reticulated and sometimes vacuolated, much resembling that of the aflagellate forms previously described

Trophonucleus—This is an inconspicuous body an account of its faint staining but is relatively large and is usually situated somewhat to the posterior of the middle of the body of the parasite In its interior there is sometimes to be made out a more darkly staining mass presumably the karyosome and this, not infrequently, takes a rod-like shape

Parabasal—This body, as in previously described forms, stains very conspicuously It is usually ovoid and is situated near and anteriorly to the trophonucleus From it springs the flagellum and, in the larger forms, the undulating membrane

Flagellar vacuole—In some of the longer forms (Plate XXXIV, fig 12), there appears to be a distinct flagellar vacuole on the anterior circumference of which lies the parabasal As this is not always present, it may merely represent one of the vacuoles already mentioned as occurring in the cytoplasm but, when present, it is usually well defined and large and its presence in the situation described is at least suggestive

Flagellum and undulating membrane—In the shorter forms the flagellum only is present It arises from near the parabasal and the intracytoplasmic portion or axoneme is often visible It thickens at the surface of the parasite due to its taking on a periplastic covering In the earlier stages of crithidial formation the rudimentary flagellum is short and straight or slightly curved but rapidly increases in length and pliability

The undulating membrane, which is present in the fully matured crithidial forms, is not a conspicuous structure, but, under suitable conditions, can generally be distinguished It is very narrow and not thrown into folds as is the case with that of the adult trypanosome

The blepharoplast—This, as a rule, is not in evidence in specimens stained with iron hæmatoxylin owing to its small size and close approximation to the parabasal,

but in dried preparations stained with Giemsa stain it is often brought out very distinctly

(5) *Eight days after ingestion* —The period eight days has been taken arbitrarily as representing the stage at which development of the trypanosome in the sandfly is complete and the infective stage of the parasite has been produced. This stage, however, may be reached earlier, or full development of infection may take longer to be completed although this is unusual. At this stage the infection, as already described in the life-history, is still manufacturing migratory hordes of the free swimming parasites described in the last section which make their way down the hindgut to the rectum and the result of their active division there is a massive growth of short forms. It is these short forms, presumably the infective stage of the parasite, which we now propose to describe.

Infective forms of T. phlebotomi —(Plate XXXIV, fig. 14) These forms are spherical, ovoid or pyriform, of small size, measuring on an average 2.6μ by 2.1μ and with markedly vacuolated cytoplasm. The parabasal is disproportionately large and takes an intense stain, but the trophonucleus stains very faintly and may be almost invisible.

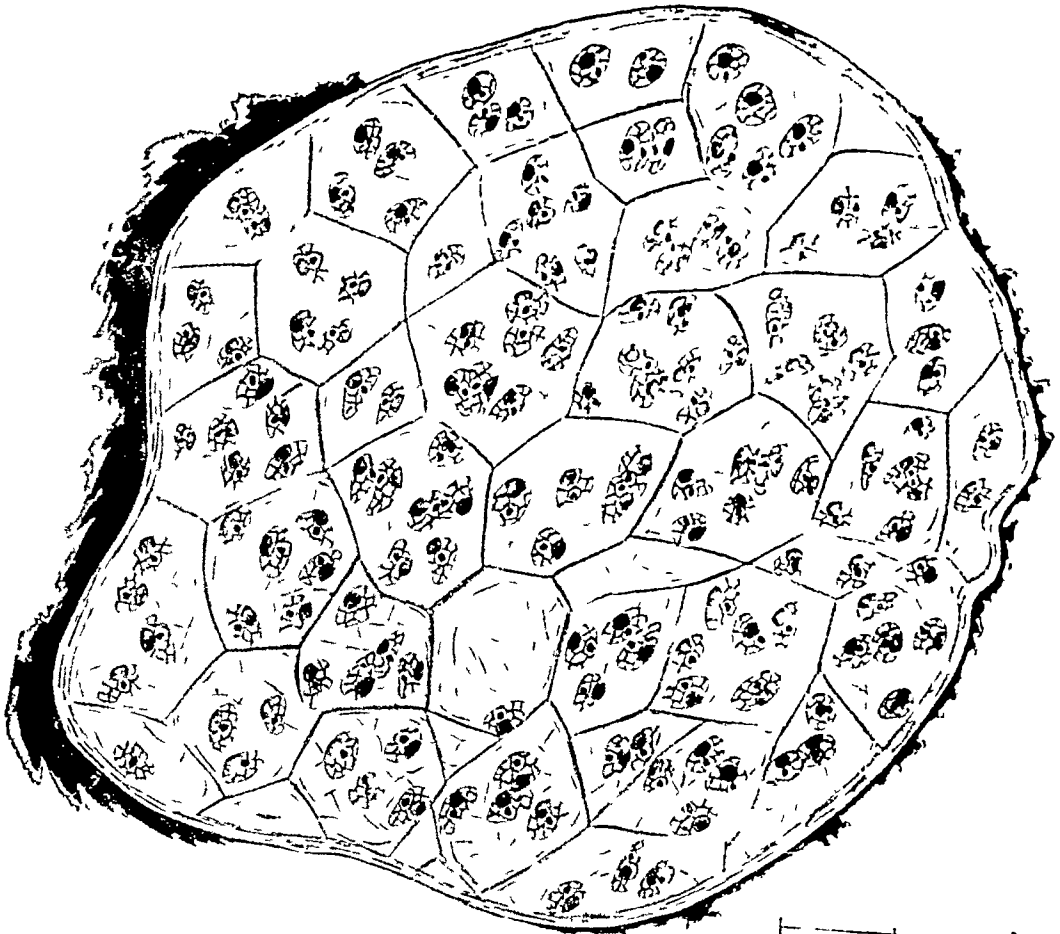
Some individuals show a rudimentary flagellum, but many appear to be quite aflagellate. Owing to the small size of the individuals, we have not been able to determine any details of internal structure beyond those given above.

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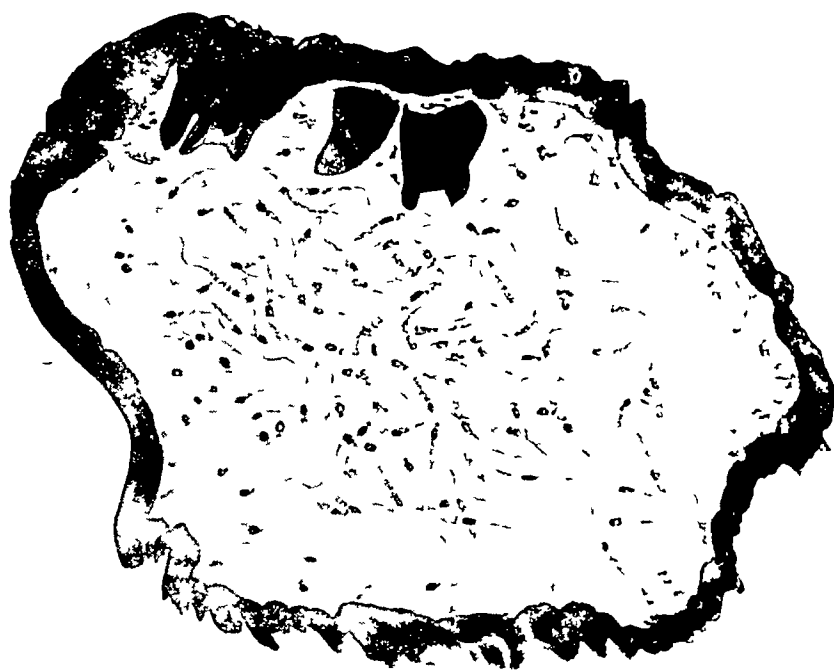
EXPLANATION OF PLATE XXXI

- Fig 1 Section of primary cyst of *T phlebotomi* in midgut of *P minutus* var *shorti*,
twenty-four hours after its feed on *H frenatus* Note commencing
meshwork
- „ 2 Section of primary cyst, forty hours after feed Note fully developed mesh-
work



EXPLANATION OF PLATE XXXII

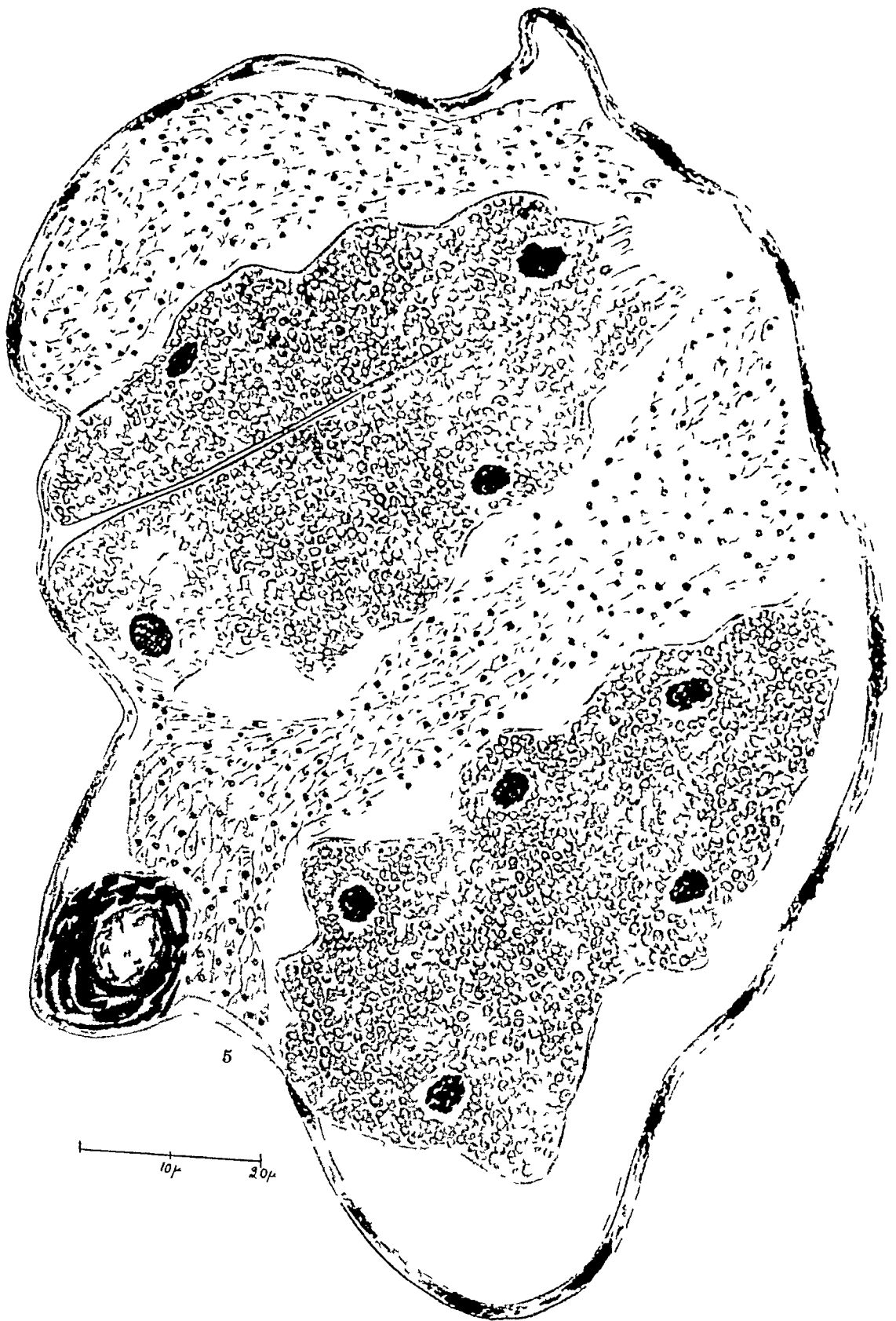
- Fig 3 Section of primary cyst, seventy-two hours after feed Note secondary cysts
containing flagellates
- „ 4 Section of crithidial flagellate forms of *T phlebotomi* in rectum of *P minutus*
var shortii



10μ 20μ

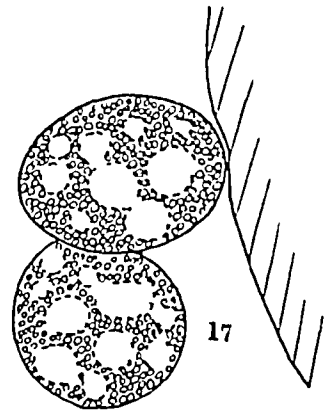
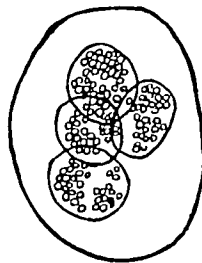
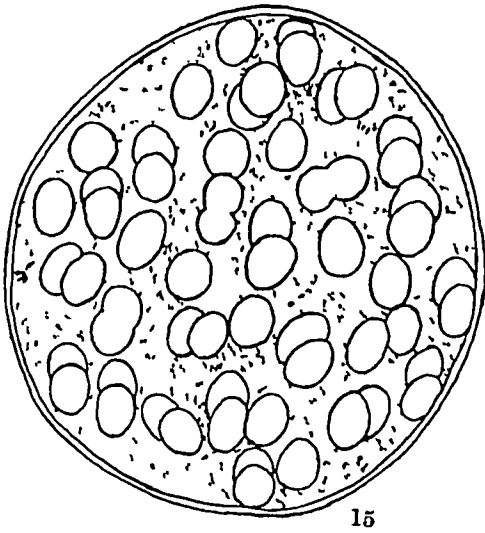
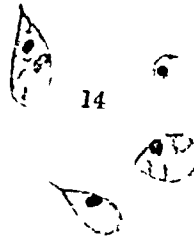
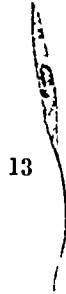
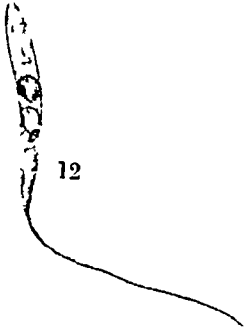
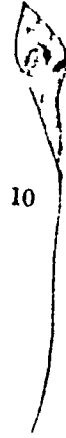
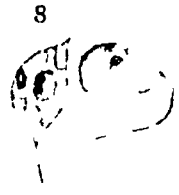
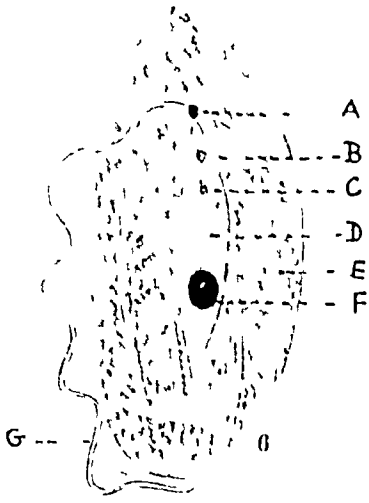
EXPLANATION OF PLATE XXXIII

- Fig 5 High power appearance of section through rectum of *P. minutus* var *shortti* eight days after first feed. Note 'metacyclic' forms of *T. phlebotomi* filling spaces between the two rectal papillæ.



EXPLANATION OF PLATE XXXIV

- Fig 6 Adult form of *T phlebotomi* in blood of *H frenatus*
 A Parabasal, B X-granule C Third granule, D Axostylar body,
 E Myonemes, F Trophonucleus, G Undulating membrane
 „ 7 Dividing form of *T phlebotomi* showing delayed division of trophonucleus
 „ 8 Early stages in the formation of crithidial forms of *T phlebotomi*
 Figs 9 and 10 Immature crithidial forms of *T phlebotomi*
 Fig. 11 Fully developed crithidial form of *T phlebotomi*
 „ 12 Do showing flagellar vacuole
 „ 13 Small crithidial form of *T phlebotomi*
 „ 14 'Metacyclic' forms of *T phlebotomi* from rectum of *P minutus* var *shorti*
 „ 15 Line drawing of twenty-four-hour cyst of *T phlebotomi* in fresh preparation
 „ 16 Line drawing of four-individual stage of primary cyst in fresh preparation
 „ 17 Line drawing of earliest noted predivision forms of *T phlebotomi* in fresh preparation



10μ 20μ

10μ 20μ

EXPLANATION OF PLATE XXXV

- Fig 18 Microphotograph of section of twenty-four-hour primary cyst of *T phlebotomi* in midgut of *P minutus* var *shortti*
- „ 19 Microphotograph of median sagittal section of *P minutus* var *shortti* showing position of two primary cysts in midgut (forty-hour stage)
- „ 20 Do Higher magnification to show trabeculae in cysts
- „ 21 Microphotograph of section of seventy-two-hour primary cyst of *T phlebotomi* in midgut of *P minutus* var *shortti* Note marked trabecular structure
- „ 22 Microphotograph of median sagittal section through terminal abdominal segments of *P minutus* var *shortti* showing position of flagellates in rectal ampulla (one hundred and thirteen hours)
- „ 23 Microphotograph of median sagittal section of terminal segments of *P minutus* var *shortti* eight days after initial feed Note two masses of 'metacyclic forms of *T phlebotomi* lying in rectal ampulla between the rectal papillae
- „ 24 Microphotograph of section of primary cyst in midgut of *P minutus* var *shortti* eight days after initial feed showing multinucleate aggregations of secondary cysts

18



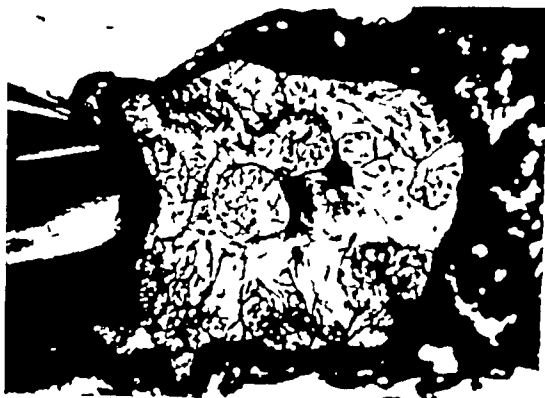
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OBSERVATIONS ON ANÆMIA IN PATIENTS WITH ENLARGED MALARIAL SPLEENS

BY

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[Received for publication, June 5, 1931]

IN the production of anæmia in certain patients whose spleens have become enlarged as a result of chronic malaria causes other than the direct destruction of red blood cells by the parasites, but nevertheless connected with malaria, would seem to be operative. Severe anæmia sometimes occurs in individuals with splenic enlargement in whose peripheral blood no parasites can be found and who have not suffered from active malaria for months. In many of these there is no evidence of any other disease. Excessive hæmolytic activity of the enlarged spleen, especially when the corpuscles are abnormally fragile (Hughes and Shrivastava, 1927), and damage to the bone marrow from malaria are possible causative factors. In some patients the effects of these may be aggravated by dietetic deficiencies, by the strain of pregnancy and lactation and by chronic diseases such as ankylostomiasis, tuberculosis, syphilis, dysentery, etc. Bignami (1912) drew attention to the occurrence of progressive anæmia in malarial subjects after the cessation of the malarial fever and termed it post-malarial anæmia. He was of opinion that it was induced by age, malnutrition, overwork, pregnancy, nursing, etc., and that it was not due merely to malarial infection. He divided the cases into four types according to the characters of the blood.

This paper is based on a study of anæmia in 31 chronic malarial patients (all males) who were admitted to the Mayo Hospital, Lahore, on account of gradually increasing weakness intensified in many instances by the discomfort caused by a very

large spleen In some the liver was enlarged as well as the spleen and in one there was hepatic enlargement only No active intercurrent disease was present in any of the cases except one (Table I, No 1) at the time they came under observation * They all had a history of repeated attacks of 'fever' which was treated insufficiently or not at all, but only 11 had a temperature while in hospital In the majority there had been no fever for weeks previous to admission Malarial parasites were not found in the blood of any patient Observations included enumeration of the total red cells, the reticulocytes (by Todd's method) (Todd, 1923) and the leucocytes, differential counts, Arneeth counts, estimation of hæmoglobin by the Newcomer method (normal 15 grammes per 100 c c blood), estimation of plasma bilirubin, determination of the fragility of the red blood cells by Orahovats' method (1926) and of their average diameter by Eve's halometer The urine was tested for urobilin by Ehrlich's and Schlesinger's methods The examination was more complete in some patients than in others

The cases fell into two groups In one group, which consisted of 17 individuals, all with enlarged spleens, there were slight or moderate changes in the morphology and staining characters of the red cells which numbered from $2\frac{1}{2}$ to $4\frac{1}{2}$ millions per c mm The hæmoglobin varied from 30 to 70 per cent of the normal and the colour index averaged 0.7 The mean size of the corpuscles was normal and nucleated red cells were scanty Megaloblasts were not seen Poikilocytosis was absent or present to a small extent The reticulocytes ranged from 2 to 10 per cent Corpuscular fragility was normal in all except three individuals in whom it was decreased In every patient there was a greater or less degree of hyperbilirubinæmia and of urobilinuria The bile pigment was of the 'indirect' type In the other group (Table I), which consisted of 14 cases, the blood picture showed marked departures from the normal Some or all of the following features were seen in every patient examined marked poikilocytosis, polychromasia, punctate basophilia, hypochromasia and anochromasia (heap-up of the hæmoglobin at the periphery of the corpuscle leaving the centre pale) In some individuals there was great diminution in the red cells and in the hæmoglobin In three the colour index was very low while in one it was abnormally high In the remainder it was the same as in the first group The reticulocytes varied from 34 per cent in Nos 13 and 14 to 1 per cent in No 6 Normoblasts were present in all cases except two and megaloblasts in most The average diameter of the red cells was increased in six and normal in eight Corpuscular fragility was greater than normal in three With one exception all these patients had enlarged spleens and increased amounts of bilirubin in the plasma The exception was No 11 whose spleen could not be felt but whose liver was enlarged $1\frac{1}{2}$ inches below the costal margin In this case the plasma bilirubin was normal or slightly raised, nucleated

* The Wassermann test was carried out in almost all cases and was negative in every instance

TABLE I

No	Date	Name and age	Clinical notes	R B C per c mm (millions)	Hemoglobin (per cent of normal)	Colour index	Average size of red cells (microns)	Fragility of red cells in saline *	Plasma bilirubin in van den Bergh units	Urobilin in urine	Reticulocytes (per cent of red cells)	W B C per c mm	Differential counts †	Abnormalities of red cells	REMARKS
1	6-12-30	N G 35 years	History of malarial fever off and on. Edema all over the body. Spleen 2½ inches below costal margin. Albumin in urine. Heart sounds very weak. B P 80/60. Died on 10-12-30 of heart failure. No malarial parasites found.	1.65	12	0.35	8.10	D	2.8	++	4	6,400	L 44.0 W 37 P 32.0 E 0.3	Hypochromasia, anisochromasia, poikilocytosis, anisocytosis, diffuse polychromasia, normoblasts and megaloblasts	Armet count 20, 32, 26, 18, † Post-mortem completely adherent pericardium. Fibrosis of myocardium. Plastic peritonitis and double adherent pleurisy. Haemorrhage in liver, spleen, kidney and other organs. Red marrow markedly increased. Microscopically the spleen showed changes indicative of chronic malaria. Marrow megaloblastic. No malarial parasites found in marrow, spleen or liver.
2	16-12-30	A 20 years	Over a year ago had malaria for about 3 months. Spleen became enlarged. This year (two months ago) he again got fever and spleen became more enlarged. Spleen now 9 inches and liver 2½ inches below costal margin. No malarial parasites found.	3.02	50	0.83	7.10	N	2.7	++	7	6,470	L 42.0 W 20.7 P 37.0 E 0.3	Some poikilocytosis, anisocytosis, diffuse polychromasia, punctate basophilic normoblasts and megaloblasts	

* D—Decreased fragility

N—Normal

I—Increased

† L—Lymphocytes

M—Monocytes

P—Polynuclears

E—Eosinophiles

Wye—Wyeocytes

TABLE I—*contd*

No	Date	Name and age	Clinical notes	R B C per c mm (millions)	Hemoglobin (per cent of normal)	Colour index	Average size of red cells (microns)	Fragility of red cells in saline *	Plasma bilirubin in van den Bergh units	Urobilin in urine	Reticulocytes (per cent of red cells)	W B C per c mm	Differential counts †	Abnormalities of red cells	REMARKS
3	28-9-30	S K 30 years	Spleen enlarged since chld hood Had an attack of fever and jaundice two years ago when spleen and liver became more enlarged, and remained so A month ago there was another attack of fever followed by jaundice Spleen now 7 inches and liver 2½ inches below costal margin No fever in hospital No malarial parasites found	1.80	25	0.69		N	4.7	+		5,000	L 31 M 6 P 62 E 1		
4	11-10-30	L. S 50 years	History of malaria of long duration 6 years ago Spleen was enlarged 3 years ago Spleen now 10 inches and liver 4 inches below costal margin No fever in hospital No malarial parasites found	1.89	20	0.54	7.32	N	2.1	+		4,000	L 49 M 4 P 46 E 1	Poikilocytosis, a few megaloblasts and normoblasts, anisocytosis and anochromasia	
5	26-11-30	A. R. 30 years	Has been getting fever for 3 months Spleen 4 inches and liver 1½ inches below costal margin Systolic murmur localized to mitral area Slight enlargement of heart. No malarial parasites found	1.84	31	0.86	7.32	I	2.4	++	16		L 40 M 4 P 55 E 1		

6	22-1-31	T R 30 years	History of malarial fever off and on for the last two years Spleen 6 inches and liver just palpable below the costal margin Developed pneumonia on 25-1-31 and died on 27-1-31 No malarial parasites found	134	20	0.77	7.64	N	31	++	1	5,500	L M P E	53.3 37 43.0 0.0	Poikilocytosis, anisocytosis, diffuse polychromasia, punctate basophils, anochromasia, normoblasts and megaloblasts	Arcth count 20, 32, 18, 4 Post-mortem secondary broncho-pneumonia, Bone marrow megaloblastic
7	8-4-31	M D 30 years	Two years ago suffered from malarial fever lasting about two months Spleen and liver became enlarged three months ago after another attack of fever Spleen now 2 inches and liver 1 1/2 inches below the costal margin Some portal obstruction No malarial parasites	277	48	0.85	7.81	I	21	+	6	6,070	L M P E	27.0 20 69.7 1.3	Anochromasia, anisocytosis, poikilocytosis diffuse polychromasia and punctate basophils No nucleated red cells seen	
8	5-5-31	M G 30 years	Gives history of malarial fever occurring every autumn Spleen became enlarged about 8 months ago after an attack of fever Spleen now 7 inches and liver 2 inches below costal margin Heart enlarged Mitral stenosis Irregular low temperature (up to 99°F) for 10 days after admission No malarial parasites found	141	27	0.97	8.10	D	21	+	3	3,030	L M P E Wbc	19.0 27 16.0 1.7 10	Poikilocytosis and anisocytosis diffuse polychromasia, punctate basophils, normoblasts, megaloblasts and anochromasia	

* D — Decreased fragility

N — Normal

I — Increased

† L — Lymphocytes

M — Monocytes

P — Polynuclears

E — Eosinophiles

Wbc — Wbc's

TABLE I—contd

No	Date	Name and age	Clinical notes	R B C per c mm (millions)	Hæmoglobin (per cent of normal)	Colour index.	Average size of red cells (microns)	Fragility of red cells in saline *	Plasma bilirubin in van den Bergh units	Urobilin in urine	Reticulocytes (per cent of red cells)	W B C per c mm	Differential counts †	Abnormalities of red cells	REMARKS
9	11-5-31	M A 26 years	History of chronic malaria. Had fever last about 6 months ago, after which his spleen became enlarged. Spleen now 8 inches and liver 3 inches below costal margin. Ascites and some oedema of feet. Traces of albumin in urine. Temperature of 99°-100°F for 2 days after admission.	0.98	17	0.85	8.50	N	3.7	++	10	4,000	L 40 M 7 P 17 E 53 Mye 03 20	Poikilocytosis, anisocytosis, normoblasts, megaloblasts, diffuse polychromasia, punctate basophilia, hypochromasia and anochromasia	
10	14-1-31	R 27 years	History of chronic malaria. Feeling weak for past 7 months. Spleen 2 inches below the costal margin. Liver just palpable. No fever in hospital. No malarial parasites found.	2.14	37	0.88	7.64	N	2.7	++	16	5,600	L 35 M 73 P 53 E 13 Mye 11	Anisocytosis, diffuse polychromasia, occasional megaloblasts and normoblasts and anochromasia	Given liver, reduced iron, quinine and plasmoquine
	21-1-31			2.89	49	0.87	7.32		1.9	+	10	5,770	L 38 M 27 P 55 E 33 Mye 10	Slight anisocytosis, few normoblasts, and some diffuse polychromasia	Patient's condition improved

11	2-2-31	S 25 years	History of occasional attacks of malaria for a long time. Complains of breathlessness, fatigue and inability to work. Duration of weakness 6 years. Spleen not enlarged, liver 1½ inches below costal margin. HCl present in stomach in small amount. No parasites found.	2 21	32	0 48	8 00	D	+	+	57	11,300	L W P F Myc	267 113 520 97 03	Numerous normoblasts (16 per cent of total red cells). Poikilocytosis, anisocytosis, diffuse polychromasia and hypochromasia.	Armet count, 11, 20, 20, 10, 9, 3. Given reduced iron grs. 5 in each of 3 times a day and liver 1 lb daily.
	12-2-31			3 31	36	0 55					12	11,300	L W P F I	257 90 460 193	Abnormalities less than before.	
	24-2-31			3 79	37	0 18	7 01		+	+	6	12,700	L W P F I	170 133 537 110	Improvement continued.	
	4-3-31			4 14	42	0 51	8 20		—	+	15	9,830	L W P F E	260 113 317 110	do	
	17-3-31			4 07	46	0 58	7 22		+	±	10	9,570	L W P F	267 107 187 113	do	Quinin 5 grs. 30 daily added to liver tonic at 21-3-31 for 12 days.
	30-3-31			3 77	18	0 63	8 10		—	—	6	10,170	L W P F E	250 83 550 111	do	Radiocolum 25 v. added to liver tonic above treatment from 1-4-31.
	11-4-31			3 00	53	0 63	8 00		—	±	8	9 670	L W P F E	287 100 527 96	do	

† L—Lymphocytes
W—Monocytes
P—Polynuclears
F—Eosinophils
Myc—Mycocytes

* D—Decreased fragility
N—Normal
I—Increased

TABLE I—contd

No	Date	Name and age	Clinical notes	R B C per c mm (millions)	Hemoglobin (per cent of normal)	Colour index	Average size of red cells (microns)	Fragility of red cells in saline *	Plasma bilirubin in van den Bergh units	Urobilin in urine	Reticulocytes (per cent of red cells)	W B C per c mm	Differential counts †	Abnormalities of red cells	REMARKS
	23-1-31			4.12	59	0.67	8.1		—	—	3	9,800	L 24.3 M 9.4 P 50.0 E 16.3	Almost normal Cells still somewhat pale	
12	12-2-31	S. L. 16 years	Fever off and on for the last 3 months, some times with nausea and vomiting, headache and weakness. Spleen filling most of the abdomen. Liver 1½ inches below costal margin. History of malarial fever two years ago, since when he has been getting fever every now and then. No parasites found.	1.6	27	0.85	7.48		3.5	++	20	3,070	L 55.6 M 3.4 P 40.0 E 1.0	A fair number of normoblasts and megaloblasts, hypochromasia, anisocytosis, poikilocytosis. Diffuse polychromasia and punctate basophilia.	Arneith count 20, 44, 20, 6, 10. Given reduced iron 15 grs and liver ½ lb daily from 15-2-31.
	25-2-31			3.22	50	0.78	7.48	N	2.3	++	9	6,030	L 39.0 M 6.0 P 50.7 E 4.3	do	
	29-3-31			4.30	80	0.93	7.48		1.1	+	4.5	6,530	L 46.6 M 5.7 P 40.0 E 7.7	No nucleated red cells, no diffuse polychromasia. Other abnormalities same as before.	

10-3-31			4 17	67	0 79	7 40		+	+	4 6	6,170	L M P E	44 6 57 34 3 15 4	S o m e anisocytosis, poikilocytosis and hypochromasia. No basophils	30 grs of quinine daily started from 21-3-31. The patient left the hospital on 27-3-31 very much improved. Spleen decreased markedly during treat- ment with liver and iron only
27-3-31			4 36	78	0 89	7 32		+	+	1 8	8,500	L M P E	33 3 33 36 7 26 7	Abnormalities much less than before	
13 3-2-31	G 30 years	Frequent attacks of malaria, especially during the last two months. Spleen got gradually larger and larger till at present it fills most of the abdomen. No parasites found	3 02	43	0 72	7 64	L	3 3	++	34	7,570	L M P E Myc	45 0 53 43 4 60 0 3	A few normoblasts, diffuse polychro- masia, some aniso- cytosis and hypo- chromasia	Given reduced iron 15 grs and liver $\frac{1}{2}$ lb daily
17-2-31			2 95	41	0 68	7 91	I	4 7	++	26	6,170	L M P E	37 7 47 53 0 16	Normoblasts and megakaryoblasts, diffuse polychromasia, punc- tate basophilic, anisocytosis and some poikilocytosis	Armeth count 28, 46, 22, 4, 0
26-2-31			2 54	43	0 86	7 32		5 2	++	51	6,270	L M P E	38 3 53 48 4 80	Occasional megaloblasts, diffuse polychromasia, punctate basophilic, some anisocytosis and poikilocytosis	
9-3-31			2 31	44	0 96	7 72		5 2	++	38	5,530	L M P E	33 7 13 59 7 53	Abnormalities less	Spleen a little smaller

* D — Decreased fragility
N — Normal
I — Increased

† L — Lymphocytes,
M — Monocytes,
P — Polynuclears
E — Eosinophils,
Myc — Myelocytes.

TABLE I—*concd*

No	Date	Name and age	Clinical notes	R B C per c mm (millions)	Hæmoglobin (per cent of normal)	Colour index	Average size of red cells (microns)	Fragility of red cells in saline *	Plasma bilirubin in van den Bergh units	Urobilin in urine	Reticulocytes (per cent of red cells)	W B C per c mm.	Differential counts †	Abnormalities of red cells	REMARKS
	18-3-31			2.31	44	0.96	7.48		2.7	++	27	5,170	L. 26.0 M. 5.0 P. 62.0 E. 7.0	Improvement continued	30 grs of quinine daily added to the above treatment from 21-3-31 for ten days. No effect on spleen
	31-3-31			2.84	46	0.82	7.32		3.0	++	21	6,170	L. 38.7 M. 2.7 P. 49.3 E. 9.3	do	
	15-4-31			2.86	51	0.88	7.17		2.8	++	18	6,700	L. 34.0 M. 3.3 P. 51.3 E. 11.4	Almost normal	
14	19-2-31	M. H. 20 years	Gives a history of malarial fever one year ago. Spleen 1½ inches below costal margin, liver palpable, HCl present in stomach. No fever while in hospital. No parasites found.	1.7	44	1.29	8.00	N	+	—	35	4,670	L. 32.4 M. 11.6 P. 35.3 E. 20.7	Hypochromasia, anisocytosis, some poikilocytosis, diffuse polychromasia. No nucleated red cells seen.	Armet count 20, 40, 24, 14, 2. Treated with reduced iron grs 1b, and liver ½ lb daily
	27-2-31			2.61	31	0.60	7.81		1.1	+	5.4	5,730	L. 30.4 M. 12.6 P. 43.3 E. 13.7	do	

11-3-31	3 76	45	0 60	7 17	+	±	7 5	7,900	L M P E	22 6 7 4 61 0 9 0	Abnormalities less than before	
20-3-31	4 44	50	0 56	7 32	+	±	7 3	8,670	L M P E	34 0 4 0 42 0 20 0	do	Quinine grs 30 daily added to the above treatment from 21-3-31 for 10 days.
31-3-31	4 25	54	0 64	7 48	—	—	4 6	7,670	L M P E	29 3 4 7 45 0 20 3	Some poikilocytosis and some anisocytosis Otherwise normal	.

* D —Decreased fragility
N —Normal
I —Increased

† L —Lymphocytes
M —Monocytes
P —Polynuclears
E —Eosinophiles
Mye —Myelocytes

red cells were very numerous and included many megaloblasts, the average diameter of the erythrocytes was high (8.0μ), and the colour index was low (0.48). Two cases in this group died, one (No. 6) from secondary broncho-pneumonia and one (No. 1) from heart failure, the result of old adherent pericardium and myocardial degeneration. In both the post-mortem findings resembled in many respects those of pernicious anæmia. There were large amounts of hæmosiderin in the organs, especially in the liver, spleen and kidneys and the bone marrow in each showed a typical megaloblastic reaction. In sections of the spleen obtained from the No. 1 there were, however, in addition to the presence of iron and pigment, changes characteristic of chronic malaria, viz., proliferation of the reticulo-endothelial cells, thickening of the vessel walls and some degree of fibrosis. An Arnetz count done on No. 6 showed a shift to the left instead of the shift to the right characteristic of pernicious anæmia. The colour index was moderately low (0.77) in No. 6 and very low (0.35) in No. 1. Four severe cases remained in hospital long enough to observe the effects on the blood condition of treatment with liver and iron. The liver was mostly given raw. Fresh sheep's livers were emulsified in a small quantity of water and the 'juice' expressed through two or three layers of gauze. This was suitably flavoured and made into a 'soup,' the residue being given in sandwiches. Each patient consumed about half a pound of liver with 15 grains of reduced iron daily.

The response to this treatment was good in three individuals: the red cells and hæmoglobin increased and abnormalities in the shape and in the staining reactions of the former diminished or almost disappeared. In one of these, however, the patient whose spleen was not enlarged, the mean diameter of the erythrocytes remained above normal. In the fourth case (No. 13) there was no rise in red cells and a small increase in hæmoglobin, but the former almost regained their normal appearance. The relation between the hæmic response to treatment, the behaviour of the spleen and the amount of the plasma bilirubin in Nos. 12 and 13 is of interest. In No. 12 improvement in the blood condition (Fig. 1) was associated with reduction in the size of the spleen and of the degree of hyperbilirubinæmia. In No. 13, on the other hand, in whom there was no increase in the number of red cells as a result of treatment (Fig. 2), the spleen remained practically unaltered in size and the plasma bilirubin at first rose considerably and later fell to a small extent. The corpuscles were abnormally fragile throughout. In this patient also the reticulo-cytes did not diminish to the same extent as they did in No. 12. Administration of quinine in daily doses of 30 grains had no influence on the progress of any of the above four cases. It will be seen from the table that the changes in the red cells during treatment were not always parallel to the changes in hæmoglobin. Thus in patient No. 14 the colour index fell from 1.29 to 0.60 in eight days. This phenomenon was noticed by Williamson (1931) during the treatment of pernicious anæmia with hogs' stomach.

The total leucocytes were below normal in almost all patients in both groups, a notable exception again being No 11 in the second group, who began with a slight leucocytosis. In many cases the leucopenia was marked. There was a general

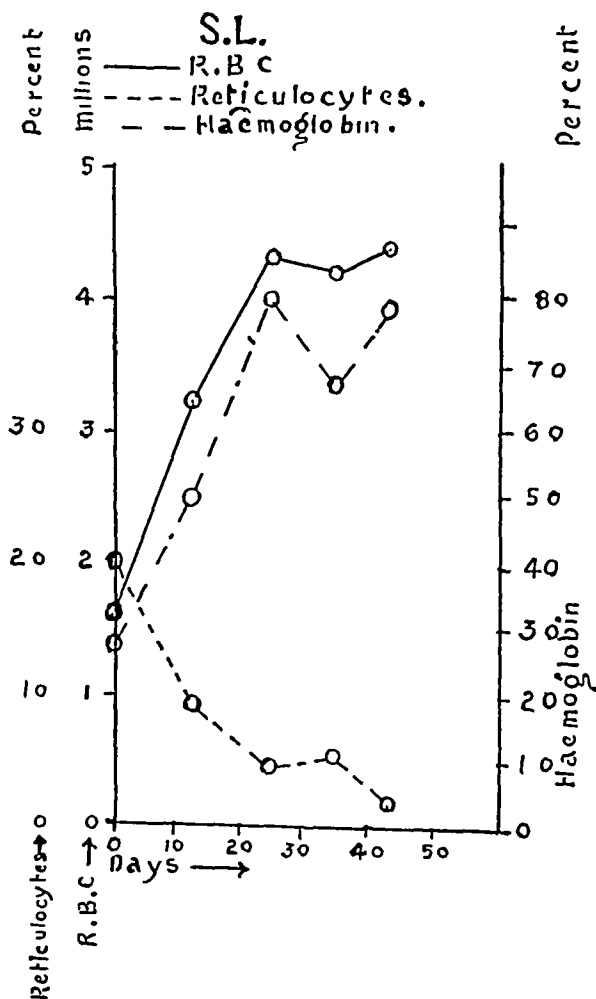


Fig. 1

tendency to a relative lymphocytosis and the monocytes were often above normal. Monocytosis was not, however, a pronounced feature. Two of the severe cases showed an eosinophilia the cause of which was not evident. Repeated examinations for hookworm eggs were negative. The eosinophiles increased with liver treatment.

in two patients. Fractional test meals were done on Nos 11 and 14 and revealed the presence of gastric HCl in each case. In No 11 the amount was small.

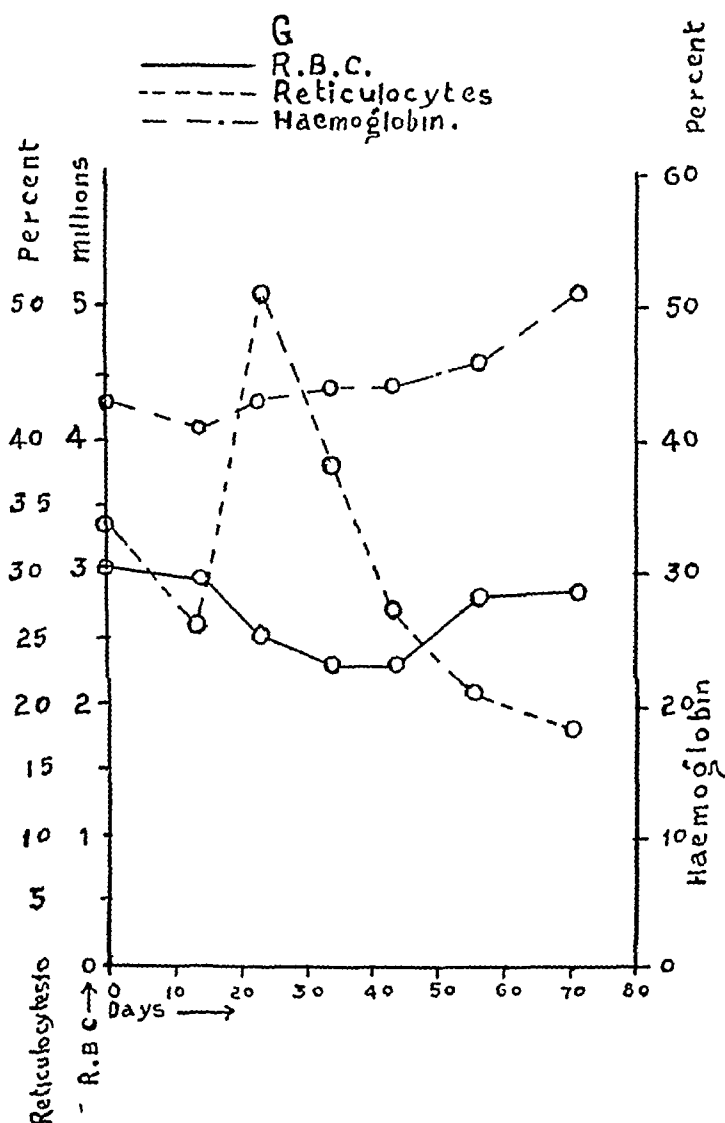


Fig 2

DISCUSSION

The bilirubin findings showed that in nearly all the patients there was excessive blood destruction. The fact that the majority had not suffered from active malaria for a considerable time makes it unlikely that this hæmolysis was due entirely or even to any great extent, to the direct action of malarial parasites. A probable cause was over-activity of the reticulo-endothelial cells of the spleen and perhaps of

other organs. The spleen in such cases often shows great hypertrophy of the cells of the pulp and the presence of large amounts of bile pigment even when no malarial parasites can be found. In many of the cases the administration of quinine in antimalarial doses did not influence the course of the anæmia and in the two that died no parasites were found in the spleen, liver or bone marrow. The findings in No. 13 indicate that in this patient the red blood cells were being destroyed as fast as they were being produced by the marrow under the influence of liver and iron. In No. 12 as the plasma bilirubin fell the spleen diminished in size although no quinine was being given at the time. A possible factor in the production of anæmia in certain individuals is increased fragility of the red blood corpuscles, a condition which was present in three patients, including No. 13, and which seems to be related in some way to activity of the spleen*. In both groups the blood showed evidence of increased marrow activity, viz., reticulocytosis, polychromasia and the presence of normoblasts. Watkins (1929) regards these features as indicative of blood regeneration in chronic idiopathic anæmia with low colour index and they have no doubt the same significance in the patients now being considered. In the second group certain of the blood changes pointed to deranged function of the marrow, viz., poikilocytosis, the presence of megaloblasts, a high colour index (one case), anochromasia, hypochromasia and, some in patients, an increased percentage of old polynuclears. Prolonged exaggerated activity may, to some extent, have been the cause of this deterioration of function as it apparently is in those cases of acquired 'idiopathic' acholuric jaundice in which the anæmia becomes Addisonian in type.

It is also possible, as already suggested, that dietetic factors played a part in the production of the severe type of anæmia. We have no exact data on the subject but we are of opinion that the diet of the classes to which these patients belonged usually contains little vitamin A and often little vitamin C. On the other hand cereals which antagonize the action of fat-soluble vitamins are largely consumed. Wills and Talpade (1930), as a result of work in Bombay, suggest that a relative deficiency in vitamins A and C, or some factor associated with such a deficiency, is concerned in the ætiology of 'pernicious anæmia' of pregnancy in that city and Wills and Mehta (1931) produced severe anæmia in Bartonella-infected rats by diets deficient in these vitamins†.

For purposes of comparison we give (Table II) the findings in three patients suffering from severe anæmia associated with pregnancy whom we were able to

* It was frequently present in a series of cases of chronic malaria with enlarged spleens which we examined in 1927 (Hughes and Shrivastava, 1927). In those the infection was active at the time of examination or had been active a short time previously.

† Wills (*Brit Med Jour*, 1931, I, p. 1059) subsequently found, however, that vitamins A and C were of no value in the treatment of 'pernicious anæmia' of pregnancy and 'tropical macrocytic anæmia,' but that in these conditions a marked curative effect, as great as that of liver extract, was produced by extract of yeast (marmite) a substance rich in the B vitamins.

TABLE II

No	Date	Name and age	Clinical notes	R B C per c mm (millions)	Hæmoglobin (per cent of normal)	Colour index.	Average size of red cells (microns)	Fragility of red cells in saline *	Plasma bilirubin in van den Bergh units	Urobilin in urine	Reticulocytes (per cent of red cells)	W B C per c mm	Differential counts †	Abnormalities of red cells	REMARKS
1	6-11-30	P 20 years	Fever of 2 months' duration. Birth of a child about 20 days ago. Much loss of blood after delivery. Breathlessness and œdema of feet after delivery. Spleen enlarged 4 inches below costal margin. Duration of enlargement not known. Liver not enlarged. HCl present in stomach.	1.46	33	1.14	7.81	N	2.4	—	15	7,530	L 25.3 M 3.0 P 71.0 E 0.0 Mye 0.7	Diffuse polychromasia, anisocytosis, poikilocytosis, megaloblasts and normoblasts	Given Pill Ferric (Bland's) grs 5 thrice daily and ½ lb liver daily. Arneth count 34, 34, 17, 10, 4
	10-11-30			3.68	55	0.74	7.64	D	+	—	8	8,100	L 59.0 M. 6.7 P 34.0 E 0.3	Some poikilocytosis, anisocytosis. B T parasites in all stages of development	
2	14-11-30	M. B 35 years	Complained of fever, breathlessness and weakness. Had a miscarriage (at 6 months) 5 days after admission. Spleen somewhat enlarged. No malarial parasites found. No fever while in hospital.	0.95	14	0.74	7.72	N	2.2	++	13	9,830	L 47.0 M 5.0 P 45.3 E 0.7 Mye 2.0	Normoblasts, megaloblasts, anisocytosis, poikilocytosis and no polychromasia	Arneth count 37, 37, 16, 5, 5. Given Pill Ferric grs 5 thrice daily and liver ½ lb daily
	20-11-30						8.49						L 50.0 M. 3.7 P 44.0 L 1.3 Mye 1.0	do	

3	30-1-31	L 30 years	Gave a history of a six months' miscarriage two months ago. Two weeks after the miscarriage she had irregular hemorrhages for some time. Present complaints are fever, cough, breathlessness on exertion and weakness. No T B in sputum. HCl present in stomach. Spleen 1½ inches below costal margin and liver palpable. No malarial parasites found.	2 96	25	0 42	7 17	D	1 0	—	9 4	8,630	L M P E	337 63 577 23	Normoblasts, megaloblasts, poikilocytosis, anisocytosis, hypochromasia, diffuse punctate basophilia and anochromasia	Armeth count 17, 38, 28, 13, 3, 1. Given quinine, plasmoquine and reduced iron grs 15 daily from 3-2-31. Put on liver extract (B W & Co) one tube daily for a week, after which given raw liver ½ lb daily.
	11-2-31	..	2 75	30	0 57	7 02	N	1 3	—	—	9 0	8,900	L M P E	280 23 683 14	A few normoblasts and megaloblasts. All the other changes as before.	Armeth count 20, 24, 34, 20, 2. Occasional myelocyte
	23-2-31		3 61	68	0 94	7 24	N	+	—	—	3	10,130	L M P E	340 23 580 50	Poikilocytosis, anisocytosis, some diffuse polychromasia, and hypochromasia	
	3-3-31		4 58	61	0 66	7 32		+	—	—	2	9,470	L M P E	283 40 640 37	Abnormalities much less than before	Left hospital on 5-3-31, very much improved.

* D — Decreased fragility

N — Normal

I — Increased

† L — Lymphocytes,

M — Monocytes

P — Polynuclears,

E — Eosinophiles,

Mye — Myelocytes

observe through the kindness of Major S N Hayes, F.R.C.S., I.M.S., Professor of Midwifery, King Edward Medical College, Lahore. One was infected with benign tertian malaria and responded well to treatment with quinine, plasmoquine, liver and iron. The colour index was 1.14. The plasma contained 2.4 units of bilirubin and the spleen was considerably enlarged. The blood showed the features of a

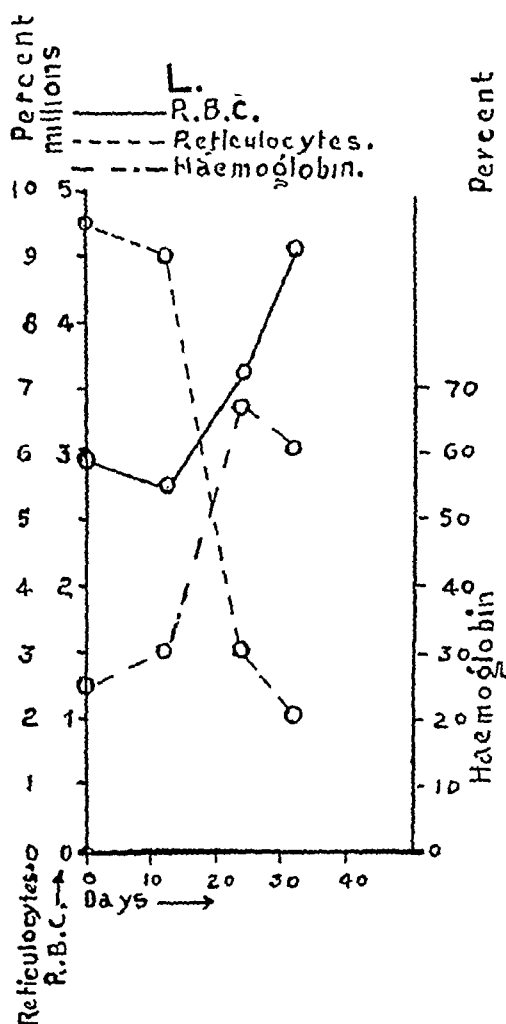


Fig. 3

severe anæmia, including megaloblasts, and the average diameter of the red cells was above normal (7.81μ). Another patient had a microcytic type of anæmia (average red cell diameter 7.17μ) with a low colour index and a blood picture indicative of deranged marrow function. The spleen was greatly enlarged and the plasma bilirubin was 1 unit. There was slight enlargement of the liver. This patient was also treated with quinine, plasmoquine, liver and iron and

showed marked improvement (Fig 3) Only one complete set of observations could be made on the third patient She was very anæmic, the red cells numbering less than a million The colour index was 0.74 and the average diameter of the corpuscles 7.72μ The spleen was moderately enlarged and the plasma bilirubin was increased to 2.2 units Gastric analyses, done on Nos 1 and 3, revealed the presence of HCl in average amounts While these three cases were all different, each resembled in its main characters one or other of the male cases described above except for the fact that No 1 was suffering from active benign tertian malaria The findings indicate that pregnancy is not the primary cause of the anæmia but is only one factor in its production

SUMMARY

A study was made of the types of anæmia seen in chronic malarial patients with splenomegaly who showed little or no signs of active malarial infection Thirty-one cases were examined

The factors concerned in the production of anæmia in these patients are discussed and it is suggested that in the milder cases the blood condition is due, to a greater or less extent, to destruction of the erythrocytes by the reticulo-endothelial cells of the enlarged spleen (and possibly of other organs), facilitated in some instances by abnormal fragility of the corpuscles

In the severe cases there were, in addition to evidences of blood destruction, signs of derangement of the function of the bone marrow This may have been the result of malaria, dietetic deficiencies or other causes Four such cases were treated with liver and iron and improvement took place in three In the fourth there was no rise in the red cells and little increase in the hæmoglobin, although there were indications of increased marrow activity In this patient the behaviour of the plasma bilirubin led to the conclusion that destruction of the corpuscles was keeping pace with their production

A description is given of three cases of severe anæmia associated with pregnancy The patients had enlarged spleens and one of them had active benign tertian malaria at the time of observation The resemblance of these cases to the others is pointed out Two, who were treated with quinine, plasmoquine, liver and iron, responded well

Our thanks are due to Major H S Anand, I M S, Professor of Physiology, Medical College, Lahore, for permission to use his laboratory and to Drs Muhamad Yusuf and K K Jaswal for assistance during the investigation Dr Jaswal supervised the preparation and administration of the raw liver

The expenses of this research were defrayed by a grant from the Indian Research Fund Association

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LEUCOCYTE CHANGES FOLLOWING INJECTION OF SANOCRY SIN IN PULMONARY TUBERCULOSIS AND THEIR SIGNIFICANCE¹

BY

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[Received for publication, June 5, 1931]

THE differential leucocyte count is of prognostic importance in pulmonary tuberculosis. A lymphocytosis occurs when healing is taking place while a lympho-pœnia, a neutrophilia and a leucocytosis are usually all bad signs. In the early stages of the disease there is some degree of polynuclear leucocytosis but in established chronic cases increase of the polynuclears indicates caseation or a mixed infection. It is also present in acute disease. A monocytosis is found when there is formation of new tubercular tissue, viz, development of endothelial and giant cells, and an increase in the 'monocyte-leucocyte ratio' is looked upon as a bad omen. The eosinophiles are increased in the allergic stage of the disease when there is good humoral and anatomic resistance (Jimez Ashua, 1921) and are scanty in the presence of acute and rapidly spreading lesions. A pleural as well as a hæmic eosinophilia follows the induction of artificial pneumothorax in phthisical subjects. This reaction seems to be characteristic of tuberculosis as it does not occur when artificial pneumothorax is produced in healthy dogs. Injection into guinea-pigs of the protein-rich exudate which sometimes forms when air is introduced into the pleural cavity in tubercular patients usually causes a rise in the circulating eosinophiles. In the absence of an exudate the local eosinophilia

* The blood changes following administration of Solganol were observed in a few instances and were found to be similar to those following administration of Sanocrysin

can be demonstrated by washing out the pleural cavity with saline (Piney, 1931) If no eosinophilia follows artificial pneumothorax the prognosis is considered bad

It is possible that these blood changes are related to the biological action of certain constituents of the tubercle bacillus Sabin and others (1930) tested biologically three products obtained from the human bacillus, viz, a lipoid fraction, a protein and a polysaccharide and found that the production of specific tubercular tissue was solely due to the lipoid fraction, the active principle of which is a saturated fatty acid called 'phthioic acid' They considered the protein and polysaccharide to be concerned in the development of an allergic state and not in the formation of tubercles The protein was shown to be highly toxic to tubercular animals

Taking the leucocyte picture to be an indication of the reaction of the body to the tubercle bacillus, a study was made of the changes in the differential count following injection of Sanocrysin in patients suffering from pulmonary tuberculosis, with a view to throwing some light on the mode of action of this substance It is now recognized that Sanocrysin does not attack the tubercle bacillus directly and that the reaction which sometimes follows its administration is not due, as was formerly supposed, to destruction of the bacilli and liberation of endotoxin Like most, if not all, so-called chemotherapeutic substances it acts in conjunction with the body cells It has been suggested that it stimulates whatever curative processes are already going on and certain clinical facts support this opinion

Observations were carried out on tubercular patients in the Mayo Hospital, Lahore, and consisted mainly in noting the blood changes at daily intervals after injections of Sanocrysin A blood examination was made immediately before each injection and in the earlier experiments two hours afterwards It was found that when reactions occurred they were maximal about 2 hours after the injections Blood for the daily counts was taken at the same time each morning while the subjects were fasting Differential counts were founded on at least 300 cells Changes in the constitutional symptoms, in the physical signs and in the sedimentation rate of the red blood cells were also observed The suspension stability of the blood, while not of diagnostic import, is usually regarded as a good indication of the progress of the disease The drug was given in doses varying from 0.05 g to 0.75 g depending on the stage of treatment, condition of the patient, etc, and increases were made very gradually Altogether the effects of 44 injections were observed in 13 phthisical patients and of 6 injections in 4 non-tubercular subjects, two of whom were suffering from chronic active malaria Figures are given only to illustrate typical features as it does not seem necessary to give details of all the observations A preliminary report on 9 injections given to 4 patients has already been published (Hughes and Shrivastava, 1930)

The effects of the injections on the total cell counts were not constant The commonest change in the leucocytes was a fall lasting 1 to 3 days Sometimes

the red cells also fell. Abrupt rises in the blood counts were occasionally seen in patients with night sweats and great fluctuations in temperature. These were probably due to concentration of the blood. As regards the differential counts, in most instances, whether the drug was beneficial or not, there was a rise in the monocytes. This usually began on the day after injection and lasted one to three days (Table I). In a few cases, in which the initial counts showed a fair proportion of these cells there was no monocytic increase (Table II). This effect was also observed in the malaria patients in whom there was a moderate initial monocytosis. When the injections gave rise to improvement an increase in the lymphocytes occurred (Table I). This generally commenced on the second day and continued for 4 to 7 days, being of longer duration with the larger than with the smaller doses. Changes similar to these were seen in the non-tubercular subjects (Table III) but the malaria was uninfluenced in the two patients who were suffering from that disease. In tubercular individuals in whom the gold salt had a bad effect there was no increase in the lymphocytes or else a fall was produced (Table IV). An increase in eosinophiles was a frequent result of the injections. The blood changes after an interval of 2 hours were a fall in the total leucocytes, in the lymphocytes and in the monocytes with a relative increase in the polynuclears.

TABLE I

(Note — *Figures within brackets indicate percentage differential counts*)

Name	Date	R B C per c mm (millions)	W B C per c mm	DIFFERENTIAL COUNT PER C MM				REMARKS
				Lympho cytes	Mono cytes	Poly nuclears	Eosino philes	
S A	31-3-30	4.74	8,230	1,424 (17.3)	494 (6.0)	6,040 (73.4)	272 (3.3)	Before injection
	do	4.39	7,130	784 (11.0)	856 (12.0)	5,154 (72.3)	335 (4.7)	2 hours after 0.25 g Sanocry- sin
	1-4-30	5.56	6,370	1,000 (15.7)	682 (10.7)	4,459 (70.0)	229 (3.6)	
	2-4-30	5.76	8,630	1,726 (20.0)	518 (6.0)	5,954 (69.0)	432 (5.0)	
	5-4-30	5.44	8,830	1,792 (20.3)	442 (5.0)	6,067 (68.7)	530 (6.0)	
	7-4-30	6.05	9,230	2,187 (23.7)	526 (5.7)	5,999 (65.0)	517 (5.6)	

TABLE I—*concl'd*

Name	Date	R B C per c mm (milli- ons)	W B C per c mm	DIFFERENTIAL COUNT PER C MM				REMARKS
				Lympho- cytes	Mono- cytes	Poly- nuclears	Eosino- philes	
	8-4-30	5 48	8,900	2,251 (25 3)	507 (5 7)	5,749 (64 6)	392 (4 4)	0 35 g injected
	do	5 33	8,030	1,229 (15 3)	514 (6 4)	5,757 (71 7)	530 (6 6)	2 hours after 0 35 g
	9-4-30	5 41	9,330	2,398 (25 7)	896 (9 6)	5,505 (59 0)	532 (5 7)	
	10-4-30	6 04	10,570	2,431 (23 0)	782 (7 4)	6,764 (64 0)	592 (5 6)	
	11-4-30	5 23	9,830	2,173 (22 1)	551 (5 6)	6,754 (68 7)	354 (3 6)	
	12-4-30	5 60	9,830	2,684 (27 3)	659 (6 7)	5,898 (60 0)	590 (6 0)	

Patient undergoing improvement Rise in lymphocytes and in monocytes

TABLE II

(Note —*Figures within brackets indicate percentage differential counts*)

Name	Date	R B C per c mm (milli- ons)	W B C per c mm	DIFFERENTIAL COUNT PER C MM				REMARKS
				Lympho- cytes	Mono- cytes	Poly- nuclears	Eosino- philes	
B	10-12-30	6 21	10,370	2,001 (19 3)	1,629 (15 7)	6,637 (64 0)	103 (1 0)	0 05 g Sanocry- sin injected Sedimentation rate high
	12-12-30	6 11	11,770	2,062 (17 6)	1,024 (8 7)	8,557 (72 7)	117 (1 0)	Sedimentation rate high
	13-12-30	6 01	10,570	1,523 (14 4)	1,120 (10 6)	7,715 (73 0)	212 (2 0)	
	15-12-30	5 64	10,300	1,237 (12 0)	1,473 (14 3)	7,416 (72 0)	175 (1 7)	do do
	17-12-30	5 81	11,370	1,479 (13 0)	1,592 (14 0)	8,073 (71 0)	226 (2 0)	

No improvement Fall in lymphocytes Initial moderate monocytosis No rise in mono-
cytes after Sanocrysin

TABLE III

(Note—Figures within brackets indicate percentage differential counts)

Name	Date	R B C per c mm (millions)	W B C per c mm	DIFFERENTIAL COUNT PER C MM				REMARKS
				Lympho cytes	Mono cytes	Poly- nuclears	Eosino philes	
A S	2-5-30	3 76	9,130	2,466 (27 0)	338 (3 7)	6,115 (67 3)	183 (2 0)	Before injection
	do	3 75	13,300	1,676 (12 6)	319 (2 4)	11,180 (84 0)	133 (1 0)	2 hours after 0.1 g Sanoery- sin
	3-5-30	4 58	11,470	3,097 (27 0)	688 (6 0)	7,491 (65 3)	195 (1 7)	
	4-5-30	4 46	9,070	2,875 (31 7)	544 (6 0)	5,505 (60 7)	145 (1 6)	
	5-5-30	4 42	9,000	2,853 (31 7)	387 (4 3)	5,463 (60 7)	297 (3 3)	
	6-5-30	4 10	8,270	3,109 (37 6)	165 (2 0)	4,772 (57 7)	223 (2 7)	

Effects of Sanoerysin in a non tubercular subject Rise in lymphocytes and in monocytes

TABLE IV

(Note—Figures within brackets indicate percentage differential counts)

Name	Date	R B C per c mm (millions)	W B C per c mm	DIFFERENTIAL COUNT PER C MM				REMARKS
				Lympho cytes	Mono cytes	Poly- nuclears	Eosino- philes	
P L	10-4-30	6 43	14,030	2,946 (21 0)	1,122 (8 0)	9,917 (70 7)	42 (0 3)	Before injection
	do	5 54	12,900	1,380 (10 7)	1,032 (8 0)	10,320 (80 0)	168 (1 3)	2 hours after 0.1 g Sanoery- sin
	11-4-30	5 06	11,630	1,117 (9 6)	1,745 (15 0)	8,489 (73 0)	279 (2 4)	
	12-4-30	5 02	10,930	1,421 (13 0)	951 (8 7)	8,416 (77 0)	142 (1 3)	
	14-4-30	5 57	12,730	1,616 (12 7)	1,018 (8 0)	9,802 (77 0)	293 (2 3)	
	15-4-30	5 44	16,730	1,958 (11 7)	602 (3 6)	14,010 (83 7)	160 (1 0)	

Patient worse after injection Rise in monocytes Fall in lymphocytes

The findings show that when injection of Sanocrysin benefits a patient suffering from pulmonary tuberculosis there are temporary changes in the circulating leucocytes resembling the changes that occur when the disease is undergoing spontaneous improvement and that similar changes follow administration of the gold salt in small doses to non-tubercular subjects. On the other hand when this substance produces a bad effect the leucocytic changes resemble those that are seen when the disease is making headway. In view of the significance of variations in the white cell picture in pulmonary tuberculosis, these facts indicate that the action of Sanocrysin in this disease consists in stimulating whatever curative processes are already going on. Its effects seem to be like that of a vaccine. It can therefore be understood why, as clinical experience has shown (Burrell, 1931, Heaf, 1929), its administration leads to improvement in those patients only whose natural forces have resisted the infection and have not been overwhelmed by the extent or progress of the disease, and why it may often turn the scales in favour of a patient in whom one lung has been collapsed when the other is slightly affected. On the other hand, it would be expected to do harm in cases who, on account of the severity of the infection, defective food or other causes, show little or no resistance. This conception of its mode of action would also explain why the effect of a small dose lasts for a shorter time than that of a large dose and why care has to be exercised in increasing the dosage. If, in patients who are showing steady improvement, each injection is given when the lymphocytosis from the previous injection is at its maximum, it is found that the spacing of doses is very much the same as that determined clinically. The temporary rise in monocytes would seem to indicate that there is nearly always an initial stimulation of the production of tubercular tissue whatever the ultimate effect may turn out to be. The blood changes which are seen 2 or 3 hours after injection, at the time when 'reactions' occur, are short-lived and differ from the slow changes above described. They support the suggestion (Burrell, 1931) that the production of a reaction and the stimulation of the tissues to kill tubercle bacilli are two different actions of the drug. Complications such as albuminuria, gastro-intestinal symptoms, erythema and rheumatic aches and pains are probably due to the direct toxic action of gold.

Although Sanocrysin has a much less specific effect in pulmonary tuberculosis than quinine has in malaria there are certain facts that suggest a resemblance between the methods of action of these drugs. It is known that quinine acts indirectly on malarial parasites, and James (1931) has expressed the opinion that this substance has a better effect on fresh malarial infections if the disease is allowed to provoke an immunity reaction by several attacks than if it is cut short early. Further, it is a matter of clinical observation that quinine has much less effect on malaria in patients whose resistance is undermined

by bad food, disease, etc, than it has in individuals who are well nourished and of good stamina

SUMMARY

A study was made of the daily blood changes following 44 injections of Sanocrysin in 13 patients suffering from pulmonary tuberculosis and of 6 injections in 4 non-tubercular subjects. It was found that --

1 When improvement in the symptoms and physical signs followed administration of the drug to tubercular patients, there was a rise in the lymphocytes which usually began on the 2nd day and continued for 4 to 7 days, according to the dose administered. A similar change was seen in the non-tubercular subjects.

2 When there was no improvement, and more especially when the effect was harmful, the lymphocytes either fell or remained stationary.

3 In all except a few instances a temporary rise in the monocytes occurred. This began on the day after injection and lasted 1 to 3 days.

In view of the changes in the leucocyte picture that occur during the natural progress of the disease, these findings suggest that the action of Sanocrysin (and similar gold preparations) in pulmonary tuberculosis is to stimulate whatever curative processes are already going on. Care should, therefore, be exercised in the selection of cases for gold treatment and the dosage should be small in the first place and very gradually increased.

Thanks are due to Major H S Anand, I M S, Professor of Physiology, King Edward Medical College, Lahore, for permission to work in his laboratory and to Drs Shujaat Ali and Sant Ram Dhall for help during the investigation.

The expenses of this research were defrayed by a grant from Indian Research Fund Association.

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CHANGES IN THE BLOOD CHEMISTRY IN OSTEOMALACIA DURING TREATMENT.

BY

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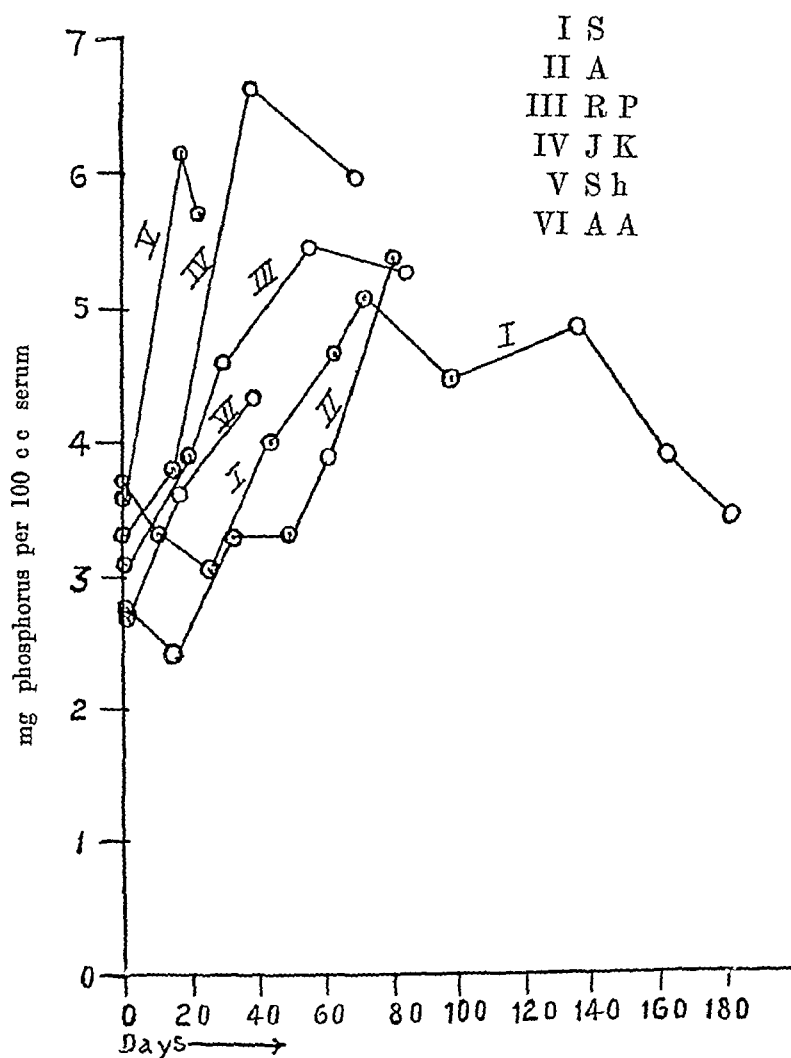
[Received for publication, May 16, 1931]

THE pathogenesis of the osteomalacia which occurs so frequently in India is similar to that of rickets, viz , perversion of the process of ossification leading to the formation of bone deficient in lime salts The blood chemistry, however, seems to vary in different cases In Bombay Wills (1931), in a series of 20 patients, found that the chief feature was a low inorganic blood phosphorus accompanied by a moderate reduction in the serum calcium This is the ordinary finding in infantile rickets Of 15 patients examined at Lahore (Hughes and others, 1929-30) 10 had a normal or slightly lowered serum calcium and a serum inorganic phosphorus which was within the adult limits of normality, 3 (who suffered from tetany) had a much reduced calcium with an inorganic phosphorus as high as that found in healthy infants and young children, one had a high calcium and a low phosphorus and one a low calcium and a low phosphorus Low serum calcium, as indicated by the occurrence of tetany, is frequent in osteomalacia in the Punjab and Kashmir Wilson (1931) found tetany in 193 out of 397 cases seen in Lahore, Amritsar and Simla and in 55 out of 135 seen in the Kangra District Spasmophilia occurs intermittently, being often brought on by pregnancy, lactation and intercurrent illnesses, and is commonest in the coldest months In China, according to Miles and Feng (1925), the blood findings in this disease resemble those in low calcium rickets The serum calcium in 10

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patients examined by them varied from 5 to 7.4 mg per 100 ccs and the inorganic plasma phosphorus from 1.8 to 3.8 mg. Wills found similar figures in one of her 20 cases in Bombay.

In the treatment of osteomalacia we have found that, whatever the initial values of the serum calcium and inorganic phosphorus, the most marked

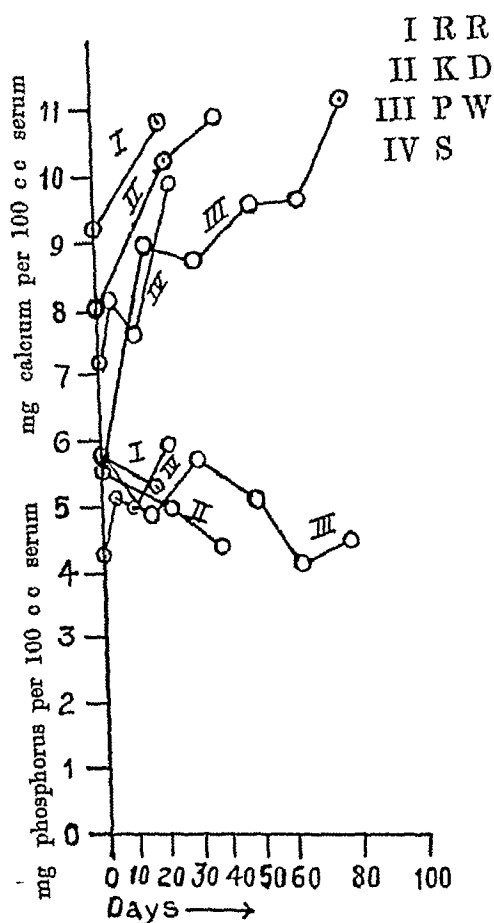


Initials refer to patients described in the table on pages 596—598

FIG 1

improvement, both symptomatically and radiologically, occurs when the former is maintained at the normal level and the latter at a height as great as, or greater than, that at which it exists in the blood of infants and young children. This is commonly stated to be from 4 to 6 mg per 100 c.c. In patients who start with a normal or slightly reduced calcium and an inorganic phosphorus at

a normal adult figure there is generally a steady rise in the latter when treatment is beneficial. A secondary fall is seen in the presence of inter-current disease and is associated with a return of symptoms. In high phosphorus low calcium cases the phosphorus sometimes drops a little at first as the calcium rises. It is interesting to note that the inorganic phosphorus of the blood attains the 'infantile' level during the healing of large fractures.



I and II are patients referred to in the table on pages 596—598
 III and IV are patients previously described
 (Ind Jour Med Res, XVIII, p 517)

FIG 2

We noticed these features during the treatment of two cases of osteomalacia last year and recently we have had an opportunity of studying them in eight other cases. The findings in these subjects are given in the table. Fig 1 illustrates the increase in the inorganic serum phosphorus in 6 patients in whom this element was originally within the adult limits of normality and Fig 2

TABLE

No	Date.	Patient	Age	Age at onset	Clinical notes	Serum calcium in mg per 100 c c of serum	Inorganic phosphorus in mg per 100 c c of serum	Treatment	REMARKS
1	3-11-30	S	26	20	Patient unable to walk Growth stunted Pelvis very much distorted Contractures in lower limbs Symptoms began after birth of first child six years ago	11.5	3.71	Ostelin	Pains less Can walk a little with assistance
	14-11-30					10.4	3.27		
	29-11-30					10.98	3.05	Radiostoleum from 4-12-30	
	17-12-30					10.8	4.1		
	5-1-31					11.04	4.7		
	14-1-31					11.14	5.1	Vigantol from 15-1-31	
	9-2-31					10.8	4.5		
	19-3-31					11.3	4.9	Cod-liver Oil and Vigantol from 26-2-31	
	15-4-31					11.3	3.96		
	5-5-31					11.4	3.45		
2	14-11-30	A	25	10	Developed pains in the feet six years ago Weakness in legs and backache occurred during first pregnancy 3 years ago Became worse after delivery Diet poor —contained hardly any milk and little ghee	11.4	2.75	Ostelin from 14-11-30	Slight improvement
	20-11-30					11.6	2.4	Radiostoleum from 4-12-30	
	17-12-30					10.8	3.3		
	3-1-31					11.6	3.3		
	14-1-31					10.8	3.9	Vigantol from 15-1-31	
	3-2-31					11.7	5.4		

Marked improvement, discharged well

3	16-12-30	R P	18	15	Symptoms began with severe backache 3 years ago during pregnancy, became worse after premature delivery—Legs contracted Diet poor—contained little milk or milk-products	91	31	Radistoleum from 16-12-30	Improvement begun
	5-1-31					100	39	Vigantol from 15-1-31	Improvement marked
	15-1-31					96	46	'Vigantol Liebertrans' from 21-1-31	Discharged well
	10-2-31					98	55	Vigantol and Cod liver Oil from 26-2-31	
	11-3-31					103	54		
4	2-1-31	J K	36	29	Married at the age of 7 Has had five children Backache started 7 years ago during one of her pregnancies, became worse after delivery and then improved Her condition became bad again during the last pregnancy 2 years ago and still worse after delivery Barely able to walk Pain over the gall bladder Diet poor	102	33	Radistoleum from 2-1-31 Vigantol from 17-1-31	Symptoms and signs of gall bladder disease No evidence of gall stones by x-rays
	17-1-31					98	38	'Vigantol Liebertrans' from 24-1-31	Improved
	9-2-31					98	66	Vigantol and Cod-liver Oil from 26-2-31	
	13-3-31					108	60		Discharged well
5	31-1-31	S H	22	21	Backache and pains in the limbs began after child birth Consumes only small quantities of milk	98	357	Vigantol from 31-1-31	Improved
	17-2-31					98	616	Vigantol and Cod liver Oil from 26-2-31	Discharged well
	12-3-31					103	574		

TABLE—*concl'd.*

No	Date	Patient	Age	Age at onset	Clinical Notes	Serum calcium in mg per 100 c c of serum	Inorganic phosphorus in mg per 100 c c of serum	Treatment	REMARKS
6	31-1-31	R R	25	19	Backache and weakness began with first pregnancy Improved after lactation Recurred with another pregnancy 4 years later Suffered from anæmic dysentery On admission had diarrhoea with histolytica cysts in the stools	9.2	5.8	Vigantol from 31-1-31	Discharged fairly well
	17-2-31					10.8	5.3		
7	6-3-31	A A	21	17	The disease began 4 years ago with mild pain in the back following miscarriage A year later after the birth of a child symptoms became worse Knee joints painful and walking difficult Reaction to treatment has been only fair Diet poor	9.6	2.7	Vigantol and Cod liver Oil from 6-3-31	Pains still present, but less Improved
	23-3-31					11.2	3.64		
	14-4-31					9.7	4.36		
8	23-3-31	K D	22	18	Pains in the back and all over the body began 4 years ago after delivery The trouble has persisted in varying degrees ever since	7.96	5.7	Vigantol and Cod liver Oil from 23-3-31	Pains less Improved
	14-4-31					10.2	4.96		
	30-4-31					10.02	4.1		

the variations in phosphorus and calcium in four patients who started with a high phosphorus and a low calcium. In subject I (see Table) there was a return of symptoms whenever the phosphorus fell below 4 mg. Serum calcium and phosphorus were estimated by the methods previously employed (Hughes and others, 1929), the serum being separated less than half an hour after the blood was withdrawn.

In addition to receiving a well balanced diet, containing fresh fruit and vegetables, the patients were given vitamins A and D in one of the following forms: Ostelin (two cases only), Radiostoleum, a special preparation of Vigantol and Cod-liver Oil (kindly supplied by Messrs Bayer-Meister Lucius) and Vigantol plus Cod-liver Oil. Calcium glycerophosphate (30 grains daily) was also given. As mentioned elsewhere, we have obtained the impression that a lack of vitamin A as well as of vitamin D plays a part in the causation of this disease. The work of Harris (1931) indicates that while vitamin D causes deposition of calcium, vitamin A, as far as bone is concerned, is essential for true osteogenesis. Wilson and Coombes (1931) found that improvement in diet (and therefore presumably increased provision of vitamin A) leads to amelioration of symptoms only when sunlight is available but that sunlight did not improve cases on deficient diet. Deficiency in vitamin C may also be a causative factor in the disease.

SUMMARY

During the treatment of osteomalacia it was observed that improvement was most marked when the serum calcium was maintained at the normal value and the serum inorganic phosphorus at or above the level at which it exists in the blood of healthy infants and young children.

Our thanks are due to Dr M Yusuf and Dr Khushdil Khan Jaswal for help during the investigation and to Major H S Anand, I M S, Professor of Physiology, for permission to work in his laboratory.

Expenses of this research were defrayed by the Indian Research Fund Association.

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ON THE FRACTIONATION OF TYPHOID IMMUNE RABBIT SERA AND THE EFFECT OF HEAT AND AGE ON THE 'H' AND 'O' TITRE OF TYPHOID CONVALESCENT HUMAN SERA

BY

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[Received for publication, May 20, 1931]

THE following experiments were designed to see what fraction or fractions of rabbit serum was responsible for the phenomenon of granular and floccular agglutination. Pooled sera of typhoid immune rabbits was fractionated with ammonium sulphate and the globulins and albumins separated in as pure a state as possible.

EXPERIMENTATION

The method employed was that of precipitation with specified concentrations of ammonium sulphate. After a preliminary observation of the agglutinating titre of the sera of three typhoid immune rabbits, the animals were bled and their serum pooled. A part of this pooled serum was kept in the frigidaire and the balance diluted with an equal volume of normal saline. To the serum thus diluted was added enough saturated solution of ammonium sulphate to bring the concentration of the resultant mixture to 66 per cent saturation. The mixture after being thoroughly shaken was filtered. The whitish precipitate thus obtained contains the 'euglobulin' fraction.

To the filtrate was then added enough ammonium sulphate to bring the concentration to 50 per cent saturation. After being shaken as before it was filtered—the precipitate now obtained being the 'pseudoglobulin'.

The filtrate obtained after the separation of pseudoglobulin was then fully saturated with crystals of ammonium sulphate and refiltered. This precipitate consists of 'albumin' fraction.

TABLE

		Type of agglutination	DILUTIONS									
			25	50	125	250	500	1,250	2,500	5,000	12,500	
Whole serum		Granular 'O '	++++	++++	++++	++++	+	0	0	0	0	
		Floccular 'H '	++++	++++	++++	++++	++++	++++	++++	++++	0	
Euglobulin		Granular 'O '	++++	++++	++++	0	0	0	0	0	0	
		Floccular 'H '	++++	++++	++++	++++	++++	++++	++++	++++	0	
Pseudoglobulin		Granular 'O '	0	0	0	0	0	0	0	0	0	
		Floccular 'H '	0	0	0	0	0	0	0	0	0	
Albumin		Granular 'O '	0	0	0	0	0	0	0	0	0	
		Floccular 'H '	0	0	0	0	0	0	0	0	0	

++++, +++, ++, + = decreasing degrees of agglutination
0 = no agglutination

The precipitates were then dissolved in 20 c.c. of distilled water and dialysed in parchmentized sacs against large quantities of tap-water for 7 to 10 days. When ammonia free each fraction was made up with normal saline to a volume equal to that of original serum.

The above table shows the agglutination titre of the whole serum and its various protein fractions.

From the analysis of the above tabulated result it would appear that the albumin and the pseudoglobulin fraction of typhoid immune rabbit sera are devoid of 'H' and 'O' agglutinins. The euglobulin fraction alone is responsible for the granular and floccular types of agglutination.

Effect of heat and age on the 'H' and 'O' titre of typhoid convalescent sera

Determination of 'O' agglutination titre has nowadays come to be recognized as a valuable aid to the estimation of degree of immunity and diagnosis of disease. This is especially true of typhoid infections in which 'O' agglutinins are said to be more closely associated with the actual disease than 'H' agglutinins.

In spite of certain limitations this method of diagnosis is becoming increasingly popular, owing to the fact that one examination of serum is said to achieve the same object as the older method of three or four examinations.

Bruce White (1930), while testing the effect of age, heat and freshness of serum on the agglutination titre of *aertryke* and *enteritidis* immune rabbit sera came to the conclusion that freshness of serum not infrequently inhibits 'O' titre when tested in the usual manner with alcohol treated suspensions of bacilli. The following experiments were designed to find out the difference, if any, in the 'O' and 'H' titre of sera of patients who had actually suffered from typhoid infection and from whose blood, faeces or urine *B. typhosus* had been recovered —

The other six convalescent sera were tested after they had been lying at room temperature for 96 hours. Both the heated and the fresh series showed practically the same 'H' and 'O' titre as when tested on the day of bleeding. Age up to 96 hours seems to make no difference in these convalescent sera as far as their agglutination titre is concerned. Inactivation, on the other hand, may have some influence on the 'O' agglutinin content. Two out of the twelve sera tested showed practically no granular agglutination in the fresh specimen while the same sera, when inactivated for 20 minutes at 55°C. exhibited 'O' agglutination up to 1 to 50. Similar tests carried out with immune sera of three rabbits showed that neither 'H' nor 'O' titre was affected by heating up to 55°C for 20 minutes.

Agglutination results of fresh and heated typhoid convalescent sera (after Bruce White)

DILUTIONS OF TYPHOID CONVALESCENT SERA																
Patient No		Type of agglutination	Fresh serum								Serum heated at 55°C for 20 minutes					
			25	50	125	250	500	1,250	2,500	25	50	125	250	500	1,250	2,500
1	1	Floccular	+++	+++	+++	++	0	0	0	0	0	0	++	0	0	0
1	1	Granular	++++	+	0	0	0	0	0	0	0	0	0	0	0	0
2	2	Floccular	+++	+	0	0	0	0	0	0	0	0	0	0	0	0
2	2	Granular	+++	+++	+	0	0	0	0	0	0	0	0	0	0	0
3	3	Floccular	+++	+++	+++	++	+	+	+	+	+	+	+++	+	+	+
3	3	Granular	+++	+	0	0	0	0	0	0	0	0	0	0	0	0
4	4	Floccular	+++	+++	+++	++	+	+	+	+	+	+	+++	+	+	+
4	4	Granular	+++	+++	0	0	0	0	0	0	0	0	0	0	0	0
5	5	Floccular	+++	+	+++	+	0	0	0	0	0	0	+	0	0	0
5	5	Granular	+++	+	0	0	0	0	0	0	0	0	0	0	0	0
6	6	Floccular	+++	+++	+++	++	+	+	+	+	+	+	+++	+	+	+

6	Granular	+++++	+++++	+++++	+	0	0	+++++	+	+	0	0	0
7	Floccular	+++++	+++++	+++++	+++++	0	0	+++++	+++++	+	++	0	0
7	Granular	0	0	0	0	0	0	0	0	0	0	0	0
8	Floccular	+++++	+++++	+++++	+++++	+	+	+++++	+++++	+++++	++	+	0
8	Granular	+++++	+++++	+++++	0	0	0	+++++	+	+	0	0	0
9	Floccular	++	++	++	0	0	0	++	+	+	0	0	0
9	Granular	0	0	0	0	0	0	++	+	0	0	0	0
10	Floccular	+++++	+++++	+++++	+++++	+	++	+++++	+	+++++	++	++	0
10	Granular	+++++	+++++	+++++	0	0	0	+++++	+	+	0	0	0
11	Floccular	+++++	+++++	+++++	+	0	0	+++++	+	++	0	0	0
11	Granular	+++++	+++++	+++++	+	0	0	+++++	+	+	0	0	0
12	Floccular	+++++	+++++	+++++	+++++	+	++	+++++	+++++	+++++	++	++	++
12	Granular	0	0	0	0	0	0	++	+	0	0	0	0

+++++, ++++, ++, + = decreasing degrees of agglutination
 0 = no agglutination

Agglutination results of fresh and heated typhoid convalescent sera after ageing for 48 hours at room temperature

Patient No	Type of agglutination	Fresh serum after 18 hours								Serum heated at 55°C for 20 minutes, age—48 hours							
		25	50	125	250	500	1,250	2,500	25	50	125	250	500	1,250	2,500		
1	Floccular	++++	++++	++++	+	0	0	0	++++	++++	++++	+	0	0	0		
1	Granular	++++	++++	0	0	0	0	0	++++	0	0	0	0	0	0		
2	Floccular	++++	+	0	0	0	0	0	++++	+	0	0	0	0	0		
2	Granular	++++	++++	+	0	0	0	0	++++	+	0	0	0	0	0		
9	Floccular	+	0	0	0	0	0	0	+	0	0	0	0	0	0		
9	Granular	0	0	0	0	0	0	0	++++	+	0	0	0	0	0		
10	Floccular	++++	++++	++++	++++	++++	++++	0	++++	++++	++++	++++	++++	++++	0		
10	Granular	++++	++++	+	0	0	0	0	++++	+	0	0	0	0	0		
11	Floccular	++++	++++	++++	0	0	0	0	++++	++	0	0	0	0	0		
11	Granular	++++	++++	+	0	0	0	0	++++	++	0	0	0	0	0		
12	Floccular	++++	++++	++++	++++	++++	++++	+	++++	++++	++++	++++	++++	++++	++		
12	Granular	0	0	0	0	0	0	0	++++	0	0	0	0	0	0		

++++, ++++, ++++, ++, + = decreasing degrees of agglutination
0 = no agglutination

++++ = decreasing degrees of agglutination
+ = decreasing degrees of agglutination
0 = no agglutination

SUMMARY

1 The euglobulin fraction of typhoid immune rabbit serum is responsible for the floccular 'H' and the granular 'O' types of agglutination

2 The albumin and pseudoglobulin fractions are devoid of 'H' and 'O' agglutinins

3 The effect of inactivation for 20 minutes at 55°C and ageing at room temperature for 48 to 96 hours was studied on the agglutination titre of 12 typhoid convalescent human sera and three typhoid immune rabbit sera. No marked difference in 'O' or 'H' agglutination titre was observed in either series

ACKNOWLEDGMENT

I am highly indebted to Major C D M Buckley, M C, P A M C, for his very kindly supplying me with sera of known cases of typhoid fever

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DESCRIPTIONS OF EIGHT NEW SPECIES OF INDIAN CULICINE MOSQUITOES

BY

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[Received for publication June 5, 1931]

***Uranotaenia hebes* sp. n.**

DESCRIPTION of ♀ *head* covered with broad flat dark-brown scales, those along eye margins appear pale when viewed in certain positions, a moderate number of upright scales scattered over the dorsal surface extending forward nearly to the eye margins, the apices of these scales only very slightly expanded. *Antenna* about the length of the proboscis, torus pale-brown, darker on inner side, clypeus brown, palpi quite short, projecting only slightly in front of clypeus, proboscis dark-brown, about the length of the abdomen. *Thorax* integument of mesonotum rather dark-brown covered with narrow brown scales and dark bristles, some lanceolate pale-brown scales over wing root and a small collection of white or creamy lanceolate scales along margin in front of wing root, some of which are fairly broad, but no line of flat scales in this position, scutellar scales small, flat, and dark-brown, as in many other species, anterior pronotal lobes covered with white lanceolate scales with silvery sheen, some broader pale or silvery scales on posterior pronotal lobes, sterno-pleura, and mesepimeron, about 4 upper and one lower mesepimeral bristles, one posterior pronotal, no spiracular, integument of pleurae pale-brown, a small dark area behind the anterior spiracle, postnotum dark-brown. *Wings* unornamented, scales dark-brown, fork cells of about equal length, the anterior longer than in some other species, wing length about 3 mm, stem of halter pale, knob dark-brown. *Legs* dark-brown unbanded, undersides of femora paler, segment 1 of fore tarsi a little longer than the last four together, segment 1 of hind tarsi very little shorter than tibia. *Abdomen* dorsum very dark-brown without markings, venter pale-brown.

Two co-type females, Nos 2557 and 2558, are in the M S I collection, Kasauli, from Nongpoh, Assam, caught in jungle on the same day and in the same spot, March 1927 (*Barraud*)

J, MR

(609)

Remarks this species appears to be nearest to *U maculipleura* Leic but differs in possessing only one small dark area on the pleurae, some pale scales in front of the wing root, shorter palpi, and much less expanded upright scales on the head

***Aedes* (*Christophersomyia* ?) *ibis* sp. n**

Description of ♀ *head* flat scaled, dorsal surface mainly covered with black scales, a patch of white ones on the nape extending forwards nearly to the eye margins in the middle line, a border of white scales to the eyes continued round to under surface, a patch of white scales on each side of the head visible in dorsal view, below this patches of black and white scales alternate, no upright scales can be seen on the nape, the head being retracted against the thorax, ocular bristles black, antenna about the same length as the proboscis, torus brown with some white scales on the inner side, first flagellar segment with dark scales dorsally, white scales beneath, shaft of flagellum dark-brown, hairs black clypeus dark-brown without scales, palpi black, about one-quarter the length of the proboscis, latter mainly black with a very distinct white marking nearer the tip than the base, on the underside and sides, dorsally it is much narrowed and does not form a very definite ring being interrupted with dark scales *Thorax* mesonotum with white lanceolate scales the broadest of which are chiefly in front of the wing roots and on the sides towards the front margin, no dark area in front of wing root, posterior part of disc denuded, a few yellowish or brownish scales remain in front of the ante-scutellar space, dense bristles over wing roots which appear yellowish or brownish, scutellar scales broad and flat, dark and pale intermixed, those on lateral lobes chiefly dark, on mid lobe mainly light, postnotum dark-brown Anterior and posterior pronotal lobes covered with white lanceolate scales, those on lower part of latter broad and flat, patches of similar broad scales below the anterior spiracle, on prosternum, larger part of sterno-pleura, upper half of mesepimeron, and small patches on coxae *Wings* white at extreme base, otherwise dark scaled, outstanding plume scales numerous and long and fairly narrow, wing length 2.5 mm, halteres with pale stems and dark knobs *Legs* anterior surface of fore femur black with a white streak on either side near the base and a large white spot in the middle of the apical half (continuous with a diagonal white marking on posterior surface), posteriorly dark at base, then a broad white area followed by a broad dark area of nearly equal width, on the apical half there is a broad white diagonal marking from the outer side to the inner, continued nearly to the knee, outer side dark at apex mid femur black except for a broad white marking anteriorly very near the apex, outer side of hind femur white except for a black ring at base, inner side white on basal half and ventrally to the tip, apical half mainly black tibiae brownish-black, the fore and hind pair pale at base beneath most marked on the hind pair, tarsi dark-brown with small pale markings at the bases of the first three segments, most pronounced

on the hind legs. The tibiae and tarsi have, when viewed in certain positions, a pale-yellowish sheen. *Abdomen* tergites black with basal white bands on segments 4 to 7 not continued to the sides, segments 1 to 7 with lateral white patches commencing basally, those on segment 2 the largest and visible in dorsal view, those on segments 3 to 7 continued diagonally nearly to the hind margin of each tergite but not produced on to the dorsum, sternites hidden.

Type female, No. 2556 (unique), in the M. S. I. collection Kasauli, from Sukna, North Bengal, 24-29 viii 28 caught in jungle (*Sobha Ram* collector).

Remarks in general appearance and character of scaling this species resembles *Aedes* (*Christophersomyia*) *annulirostris* (Theo.) but differs in the ornamentation of the legs and mesonotum. Until the male has been discovered it is not possible to say whether it should be placed in this subgenus or in *Finlaya*.

***Aedes* (*Finlaya*) *simulatus* sp. n.**

This species resembles *Aedes* (*F.*) *macdougalli* Edw. closely, but differs as follows — proboscis pale beneath from near the base to the tip except for a dark interruption some little distance from the apex, base dark for a little less than a quarter of the total length, palpi with white scaling at the tips only. hind femur with a dark interruption in the longitudinal pale line on the inner side. In *Aedes macdougalli* the proboscis is pale beneath on the basal two-thirds, the palpi have white scaling in the middle as well as at the tip, and the pale longitudinal line on the inner surface of the hind femur is continuous from base to apex.

Description of ♀ *head* covered with narrow and upright scales for the most part very dark, but there are white scales medianly forming a line from the nape to the front of the vertex, a narrow white border to the eyes, and a patch of white scales laterally at each side, ocular bristles long, appearing very pale when viewed in certain positions, clypeus dark-brown, shaft of antenna dark-brown, hairs pale. *torsus* brownish-black with some white scales on the inner side, proboscis dark-brown on upper surface, white beneath from near the base to the tip except for a dark interruption some little distance from the apex, palpi between one-quarter and one-third the length of the proboscis with white scaling at the tips, otherwise dark-brown. *Thorax* integument of thorax almost black covered with dark-brown and pale-yellow scales, the latter arranged in lines, a median line and a pair of sublateral, the latter continued from the front to the scutellum, a pair of lateral lines commencing at the posterior pronotal lobes, curving over the wing roots and terminating at the lateral lobes of the scutellum, in addition there are some pale-yellow scales immediately in front of each wing root, scutellar scales pale-yellow, broad on the mid lobe, narrow on the lateral lobes. Integument of pleurae nearly black, anterior and posterior pronotal lobes mainly covered with broad flat white scales, some narrow ones on upper border of latter, patches of broad white or creamy scales on coxae, upper part of mesepimeron, on sterno-pleura, prosternum, behind

and below anterior spiracle, 5 posterior pronotal bristles apparently no lower mesepimeral, all pleural bristles very pale *Wings* dark scaled outstanding plume scales narrowly lanceolate, wing length 3 mm *Legs* fore femur dark anteriorly except along the inner side where there is pale scaling running for nearly the whole length, on the posterior surface, as well as on both surfaces of the mid and hind pair, there is a distinct pale longitudinal line from base to apex, except that on the inner surface of the hind pair there is a dark interruption some little distance from the knee, fore and mid tibiae dark-brown with a pale line running the whole length of the anterior surface, hind pair dark-brown with a broad white marking on the underside at the base, tarsi dark-brown with a basal white ring to segment 1, apical and basal white rings over the joint between 1 and 2, and 2 and 3, on the hind leg there is also a white ring over the joint between 3 and 4 *Abdomen* brownish-black with narrow silvery white basal bands on segments 2 to 6, widening out into lateral silvery patches, segments 1 and 7 with lateral silvery patches only, sternites brown with yellowish basal bands

Type female, No 2560 (unique), is in the M S I collection, Kasauli, from Assam, Haflong, Cachar Hills, viii 1922, larva from tree-hole (*Barnaud*)

***Aedes (Aedes) yusafi* sp. n.**

This species differs in the female from all others of the subgenus known in India in having the anterior pronotal lobes completely covered with silvery white scales. It has, up to now, been confused with *Aedes (Aedes) indicus* (Theo) with which it is found in association, and the two species resemble one another in size and general appearance. There are, however, constant differences in markings and in the structure of the genitalia. Both tarsal claws on all the legs are toothed as in *Aedes (Aedes) uniformis* (Theo)

Description of ♀ *head* mainly covered with flat brownish-black scales, a patch of white scales in the middle of the vertex in front extending forwards between the eyes, a patch of similar scales at each side of the head just visible in dorsal view, apparently no upright scales. *antenna* about the length of the proboscis, torus, shaft, and hairs dark-brown, clypeus, palpi, and proboscis brownish-black, palpi about one-fifth the length of the proboscis. *Thorax* integument of mesonotum and scutellum brownish-black, scales rather lighter in colour especially at the sides and on front margin, scutellar scales narrow and golden-brown, integument of pleurae black, anterior pronotal lobes covered with silvery white scales, patches of similar scales behind anterior spiracle, on upper and lower parts of sterno-pleura, upper part of mesepimeron, and on prosternum and coxae, posterior pronotal lobes apparently unscaled, postnotum and knobs of halteres nearly black, stems of latter pale-brown, 6 posterior pronotal bristles, about 15 upper mesepimeral but none on the lower part of this sclerite. *Wings* dark scaled, outstanding plume scales narrow, wing length 2.7 to 3 mm. *Legs* femora mainly yellowish-brown, tibiae and tarsi

brown *Abdomen* tergites almost black with rather large basal lateral white patches extending on to the dorsum but not forming complete bands, 1st tergite entirely dark, sternites with basal white bands and apical dark bands of about equal width *Hypopygium* (Plate XXXVI, fig 3) atrium and associated chitinizations comparatively small compared with the size of the cerci, post-genital plate a single lobe, not usually emarginate at the apex (in one specimen out of five examined it is slightly emarginate), cowl with a lobe on each side and a marked median depression

Type female, No 2555, is in the M S I collection, Kasauli, from Delhi, Roshanara Gardens, iv 1914 (*S R Christophers*), also 15 other females taken at the same time and place, one female from Amritsar, Punjab, viii 1910 (*S R Christophers*), one female from Karnal, Punjab, viii 1928 (*Barraud*)

I have named this species after Mohamed Yusaf, Laboratory Assistant, who drew my attention to the structure of the genitalia when making a large number of preparations for me

Aedes (Aedes) agrestis sp. n.

This species resembles *Aedes (Aedes) hirsutipleura* Barraud, both in markings and in the presence of numerous bristles and hairs on the mesepimeron, but the two species are distinct in the structure of the genitalia

Description of ♀ *head* mainly covered with almost black flat scales, a very narrow pale border to the eyes and a patch of white scales at each side just visible in dorsal view, a few upright scales on the nape, antenna about the length of the proboscis, torus brown, darker on inner side, shaft of antenna black, clypeus dark-brown, palpi black, proboscis brownish-black, palpi only about one-seventh the length of the proboscis *Thorax* mesonotum deep reddish-brown (in *Aedes hirsutipleura* Barraud this part is almost black), covered with narrow scales of the same colour a few lighter scales around the front margin, scutellum dark-brown with a few reddish-brown narrow scales, postnotum rather lighter-brown, halteres with pale stems and dark knobs as in many other species, pleurae dark-brown with patches of pale flat scales on upper part of sterno-pleura and in middle of mesepimeron, anterior and posterior pronotal lobes apparently without scales, 5 large posterior pronotal bristles and some smaller, very numerous bristles and hairs covering the larger part of the mesepimeron as in *Aedes hirsutipleura* Barraud (Barraud, 1928), on the sterno-pleura there are a number of bristles and scales at a point opposite the lower corner of the mesepimeron *Wings* dark scaled, outpale beneath *Abdomen* tergites black with small lateral white patches commencing basally and not produced on to the dorsum, sternites pale-brown *Hypopygium* (Plate XXXVI, fig 1) comparatively large, cowl only slightly curved, post-genital plate large and not markedly emarginate on the apical border

Type female, No 2553, is in the M S I collection, Kasauli, from the Nilgiri Hills, ix 1915 (*Khazan Chand*), one other female from Nagaiyah, Bombay, Deccan, viii 1921 (*Barraud*)

***Aedes (Aedes) clavatus* sp. n**

This species is very distinct in the structure of the genitalia from any other known Indian species

Description of ♂ head mainly covered with dark-brown flat scales those along the eye margins and at the sides light-brown (not white as in many other species), fairly numerous upright scales on the nape antennal hairs dark-brown or black, shaft very pale between verticils, torus brown, darker on inner side, clypeus brown, palpi and proboscis dark-brown, palpi exceeding the clypeus by a little more than its length and about one-sixth the length of the proboscis *Thorax* mesonotum chestnut-brown with a slight reddish tinge, fairly numerous black bristles, scutellar scales sparse, narrow and dark-brown, postnotum rather lighter-brown than the scutellum, integument of pleurae lighter-brown than the mesonotum, anterior and posterior pronotal lobes apparently devoid of scales, some patches of broad, flat, pale scales on the upper and lower parts of the sterno-pleura and on upper part of mesepimeron, 6 posterior pronotal bristles, 10 strong upper mesepimeral, 6 smaller arranged in a row extending downwards and within the patch of scales *Wings* dark scaled, outstanding plume scales fairly broad but few in number, wing length about 2.8 mm *Legs* dark-brown, femora paler ventrally *Abdomen* dorsally brownish-black with small basal lateral pale patches not produced on to the dorsum, venter pale-brown *Hypopygium* (Plate, XXXVI, figs 5 to 8) the coxite (side-piece) terminates in a short finger-like process on the dorsal side and there is a pointed process below the apex ventrally, style (clasper) arising from the inner side of the coxite some distance below the apex, chitinizations of proctiger (anal segment) represented by two arm-like processes, one longer than the other, both slightly clubbed and truncated at the tip

Type male, No 2197, and two other males, are in the M S I collection Kasauli, all from Sukna North Bengal, 24-29 viii 1928 (*Sobha Ram* collector)

***Aedes (Aedes) abditus* sp. n**

A small blackish species which can only be identified with certainty by examination of the genitalia In the structure of these parts, and in the presence of toothed hind claws, it most nearly resembles *Aedes (Aedes) uniformis* (Theob)

Description of ♂ head mainly covered with dark-brown, flat scales, a narrow border of pale scales to the eye margins and a patch of similar scales at each side, a few upright scales on the nape tori and clypeus brown, flagellum of antenna

black, the tip reaching some little distance beyond the end of the proboscis, palpi and proboscis dark-brown, the former about one-fifth the length of the latter. *Thorax* mesonotum and scutellum brownish-black, denuded, postnotum black, stems of halteres pale, knobs black, pleurae dark-brown with some pale flat scales on upper part of sterno-pleura, a patch of similar scales in the middle of the mesepimeron, the lower part of this sclerite without bristles or scales. 4 long posterior pronotal bristles. *Wings* with brown scales, outstanding plume scales rather narrow, wing length 2.6 mm. *Legs* dark-brown, femora pale ventrally, claws of hind tarsi toothed. *Abdomen* tergites almost black, lighter at extreme lateral edges, but there are no distinct pale patches, venter brown. *Hypopygium* the appearance of this is shown in Plate XXXVI, fig. 4. This may be compared with the drawing of similar parts of *Aedes (Aedes) uniformis* (Theo.) previously published in this Journal (Barraud, 1928).

Type female, No. 2192 (unique), is in the M. S. I. collection, Kasauli, from Sukna, North Bengal, 24-29 viii 1928 (*Sobha Ram* collector).

***Aedes (Aedes) comatus* sp. n.**

This species resembles *Aedes (Aedes) insutipleura* Barraud closely in markings and in the presence of a fair number of bristles on the lower part of the mesepimeron, but these are less numerous, and there are differences in the structure of the hypopygium.

Description of ♀ head mainly covered with brownish-black flat scales but there is a narrow border of pale scales to the eyes widening out into a fairly large patch at each side just visible in dorsal view, fairly numerous upright scales on the nape, no pale scales in the middle of the vertex, antenna a little shorter than the proboscis, torus brown, darker on inner side, flagellum black, clypeus, palpi and proboscis dark brownish-black, palpi about one-fifth the length of the proboscis. *Thorax* integument of mesonotum and scales deep chestnut-brown a few lighter scales round the anterior margin, scutellum dark-brown with a few golden-brown narrow scales, postnotum lighter-brown than the scutellum, knobs of halteres very dark, stems pale, pleurae lighter-brown than the mesonotum, patches of flat white scales on upper and lower parts of sterno-pleura, in the middle of the mesepimeron and on coxae, very few if any, scales on anterior and posterior pronotal lobes, 5 posterior pronotal bristles, about 10 bristles just below the patch of scales on the mesepimeron but not continued to the lower border of the sclerite. *Wings* dark scaled, outstanding plume scales rather narrow, wing length 3.4 mm. *Legs* dark-brown, femora pale ventrally. *Abdomen* tergites brownish-black with lateral white patches commencing basally and not produced on to the dorsum, sternites almost completely hidden, but apparently with basal pale bands. *Hypopygium* (Plate XXXVI, fig. 2) post-genital plate with pronounced lobes, cowl tri-lobed

(differing from that of any other known Indian species), atrial plates fairly large and rounded

Type female, No 2191 (unique), is in the M S I collection, Kasauli from Sukna, North Bengal, 24-29 VIII 1928 (*Sobha Ram* collector)

REFERENCE

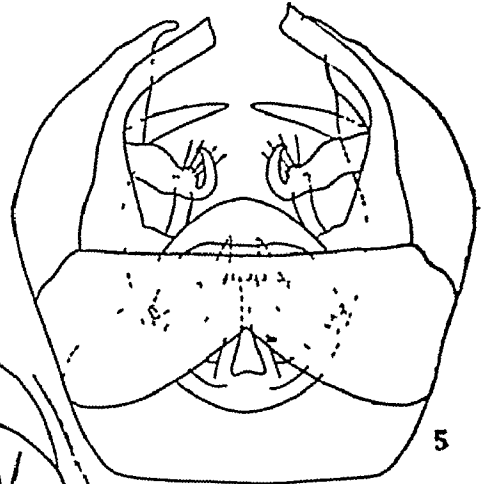
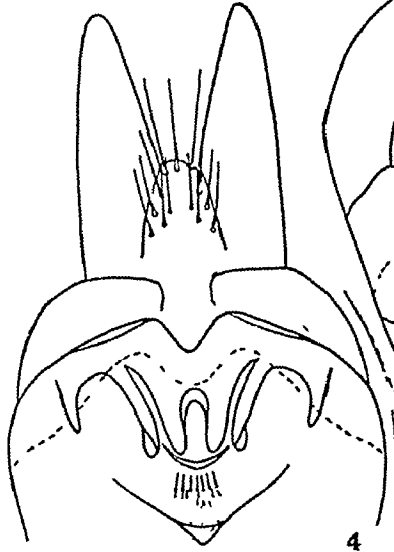
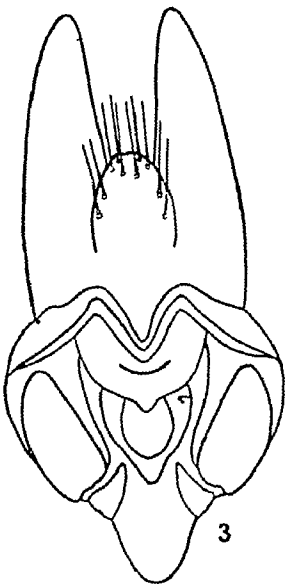
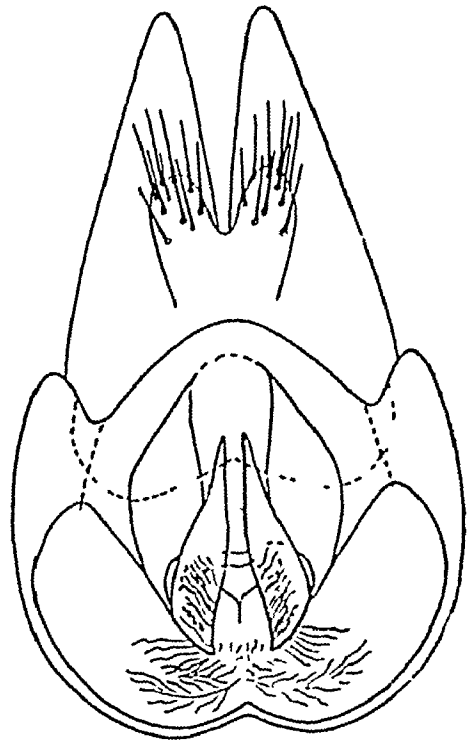
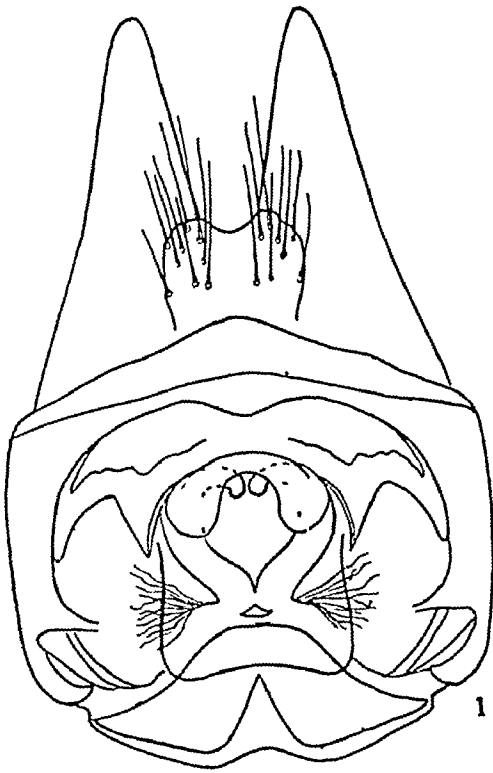
BARRAUD, P J (1928)

The Indian species of the subgenera *Skusca* and *Aedes*, with descriptions of eight new species, and remarks on a new method for identifying the females of the subgenus *Aedes* *Ind Jour. Med Res*, **16**, pp 357-375 (eight plates)

EXPLANATION OF PLATE XXXVI

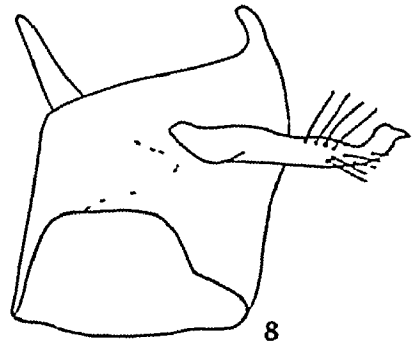
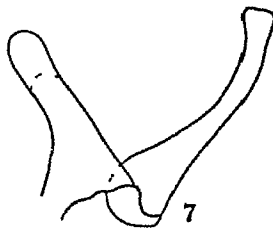
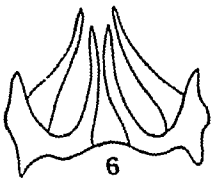
Camera lucida drawings of the genitalia of new species of *Aedes* (*Aedes*) Figures 1 to 4 drawn to the scale shown under Fig 3 Figures 5 to 8 drawn to the scale shown under Fig 5

Fig 1	<i>Aedes</i> (<i>Aedes</i>) <i>agrestis</i> sp n female	Appearance of the genitalia from beneath
„ 2	„ „ <i>comatus</i> sp n type female	ditto
„ 3	„ „ <i>yusafi</i> sp n female	ditto
„ 4	„ „ <i>abditus</i> sp n type female	ditto
„ 5	„ „ <i>clavatus</i> sp n type male	Dorsal view of the genitalia
„ 6	„ „ „	Phallosome and associated chitinizations, flat preparation
„ 7	„ „ „	Chitinizations of proctiger (anal segment), flat preparation
„ 8	„ „ „	Coxite (side-piece) and style (clasper), flat preparation



0.1 mm

0.1 mm



THE NATURAL BREEDING HABITS OF *A STEPHENSI* AS OBSERVED IN CALCUTTA

BY

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[Received for publication, June 8, 1931]

DURING the last year or two with the view of obtaining a plentiful and constant supply of *Anopheles* mosquitoes for infectivity experiments an attempt was made to keep a culture of *A stephensi* going in the laboratory and this effort at first met with some success, later on, however, the larvæ failed to develop properly and I therefore decided to study the breeding habits in nature of all the common *Anophelines* in Calcutta, and this study lasted from March to August 1930, a period including the dry and the rainy seasons

Present-day Calcutta is very extensive covering an area of 28,086 acres and can be roughly divided into three zones the city proper or the central portion, the peripheral or marginal zone formed by recent absorption of suburbs, an increase of about 60 per cent over the former area, and about 20 per cent of the population and an intermediate zone which before the extension just mentioned lay on the border zones of the old city This division is quite arbitrary, there being no sharp lines of demarcation between these areas, as one merges into the other The central portion is quite dry as the drainage system is up to date In the marginal areas there are yet water-logged places, and it will take many years to bring this area to the same standard of sanitation as the central portion In the intermediate zones the conditions are intermediate between the other two areas

There are two systems of water supply in Calcutta The filtered water for cooking, drinking and bathing purposes is usually stored in masonry vats on the ground and the unfiltered or the dirty water, which is the silt-laden water of the River Hooghly, used for flushing privies and for garden purposes, is invariably stored in galvanized iron cisterns placed on roofs of houses, the lid, if there is one, may be kept closed but it may be broken or not utilized and hence the water is left exposed Besides this water, there are some well-kept tanks throughout the whole

city On the other hand, there are still many tanks and ponds in the peripheral or marginal zones which are mostly neglected and typical of those that exist in many Bengal villages

The mosquito fauna changes as one proceeds from the central to the marginal zone In the central portion one usually obtains *A. stephensi* and to a less extent *A. subpictus* and *A. vagus* In the marginal zone there are *A. subpictus* and *A. vagus* predominating over *A. stephensi* besides these the following other species may be found *sinensis*, *barbirostris*, *fuliginosus*, *aconitus*, *funestus*, *theobaldi** (Strickland and Chowdhury, 1920)

PERMANENT BREEDING SITES

Overhead tanks —Ivengal (1920) was the first to point out that the overhead cisterns, in which the unfiltered water-supply is usually stored, harbour *stephensi* larvae and that the silt in the water is not inimical to their growth These cisterns form the permanent breeding places of this species and they can be found at any time throughout the whole year in at least one out of three defective cisterns its other favourite habitat is the masonry vats constructed by builders for soaking their bricks

Ponds and tanks —Although the tanks are well kept they are full of aquatic plants and hence at times *fuliginosus* can be found There is one long crescent shaped tank in the Eden Garden full of weeds and lilies, the edges of which are grassy and after many careful searches *fuliginosus* was found both during the dry season and during the rains in addition to *stephensi* As one approaches the outskirts of the city one finds tanks and ponds full of duckweed, and these tanks harbour nothing but *fuliginosus*, while in comparatively cleaner tanks with their inevitable grassy edges a small number of *aconitus* and *funestus* with *sinensis* and *barbirostris* can be found

Drains —On the outskirts of the city there are open earth drains where usually millions of *C. fatigans* and *D. obturbans* are commonly found, and every now and then a sprinkling of *subpictus*

The above sites one may say, constitute roughly the permanent breeding places of the anopheline mosquitoes of Calcutta

TEMPORARY BREEDING PLACES

During the rains temporary breeding places make their appearance, an account of which has been given by Basu 1930, and in these *stephensi*, *subpictus* and *vagus* are frequently met with and in a number of tanks and ponds *subpictus*, while *subpictus* along with *Culis* are found in earth drains and also at this season a fair number of *stephensi* In the small rain water puddles, in the parks and maidans, *A. vagus* and *A. subpictus* are mostly found In recent earth excavations if the

* Only one specimen has been recorded by Strickland and Chowdhury

water is clean *stephensi* is found in very large numbers and if the water is muddy *subpictus* and *vagus*

In the numerous fresh puddles that form temporarily during the dry season after a shower of rain *stephensi* is found, but if the water is foetid *subpictus* with *Culex*es and in some instance also *stephensi* are encountered

Out of the several species of Anophelines mentioned above, *stephensi* is the only dangerous species that breeds in the city proper and hence observations will be confined on the breeding habits of this species alone

FOOD OF THE LARVÆ

Of the various physical factors which contribute to the growth of mosquito larvæ the most important is the nature and character of the food supply Larvæ are provided with mandibulate mouth-parts and their food may be of the nature of hard organic substances Some of the natural breeding places of *stephensi* were examined physically and microscopically and the food material present was found to be of the nature of green algæ and organic substances, e.g. dust bits of dried leaves, dried grass, clay, bits of cooked meat, bread droppings of crows and birds etc Protozoal organisms were commonly found in the water but not always In a number of overhead cisterns containing larvæ no trace of any food material could be found in the water on examination, except the silt, carried with the water and deposited at the bottom With a view to determine how far the nature and the character of the food material can influence the growth of *stephensi* larvæ the following observations were carried out in the dry and also in the rainy seasons

I *The relation of food to the growth of larva in the dry and in the rainy seasons as observed in overhead cisterns*

The nature of the food present in the water was observed and the period occupied by the larvæ to develop into pupæ was studied In the overhead cisterns placed on roofs of houses the depth of water was usually from a few inches to five feet

Dry season

Tank No	Period	Number of days	Food
1	28 4 to 12 5	15	Deposits of green algæ at the sides, water clean, deposit of silt at the bottom, dry leaves, dead insects floating on the surface
2	28 4 to 15 5	17	
3	28 4 to 16 6	19	
4	30 4 to 20 5	21	
5	30 4 to 15 5'	16	
6	30 4 to 20 5	20	

The time taken by the larvæ to pupate in the dry season, when the food consisted mostly of green algæ, was found to range from 15 to 21 days

Tank No	Period	Number of days	Food.
7	27 4 to 18 5	23	Water muddy, deposit of silt at the bottom, no apparent food material present on the surface, tank shady, no protozoal organisms present
8	15 5 to 18 6	34	
9	15 5 to 15 6	31	
10	15 5 to 12 6	28	
11	19 5 to 11 6	23	
12	6 5 to 28 6	22	

In the same season these larvæ, deprived of their proper (²) food, were observed to occupy a period of from 22 to 34 days to change into pupæ

Tank No	Period	Number of days	Food
13	25 4 to 8 5	14	Water clean, tank well shaded, bits of raw meat, straw, bread, dead insects, and other organic substances were found floating on the surface
14	25 4 to 9 5	15	
15	25 4 to 7 5	13	
16	30 4 to 16 6	17	
17	30 4 to 13 5	14	
18	7 5 to 19 5	13	

The minimum period during the dry season taken by these larvæ to pupate, as will be found from the above, ranged from 13 to 17 days when there was abundant food material, of the nature of organic substances, present on the surface

With these figures may be compared those cited below, which show the period of larval life during the rainy season, the character and the nature of the food being as above

Tank No	Period	Number of days	Food
19	4 8 to 15 8	12	The nature of the food present was the same as in Tanks 1 to 6
20	4 8 to 17 8	14	
21	4 8 to 14 8	11	
22	13 8 to 24 8	12	
23	13 8 to 25 8	13	

Tank No	Period	Number of days	Food
24	4 8 to 19 8	16	The nature of the food present was the same as in Tanks 7 to 12
25	7 8 to 20 8	14	
26	16 8 to 27 8	12	
27	16 8 to 29 8	14	
28	7 8 to 16 8	10	The conditions were identical with those of Tanks 13 to 18
29	7 8 to 16 8	10	
30	13 8 to 23 8	11	
31	13 8 to 23 8	11	

From the above it will be seen that larvæ take a much shorter time to pupate during the rainy season than in the dry season and when the food material lies at the surface or very near to the surface rather than at a depth of say 3, 4 or 5 feet. The larval period takes about 13 to 16 days in the dry season, while this period is reduced to 10 to 11 days in the rainy season.

So far the character of the food substance as affecting the growth of *stephensi* larvæ in the two seasons in their permanent breeding places has been dealt with. In the temporary breeding areas the method adopted to study the larval growth in pools during the rainy season was to keep watch for the first appearance of larvæ two to three days after a heavy shower of rain. During the dry season larvæ were transferred from some natural breeding sites to a ditch artificially made for the purpose. The period of growth in the two seasons was observed as follows —

Dry season	On an average from 10 to 11 days
Rainy season	On an average from 7 to 9 days

II *The relation of food to the growth of larvæ as observed in specially made galvanized non cisterns*

Galvanized non tanks were made to order resembling the overhead cisterns, they were filled up from 3 to 4 feet with the same unfiltered silt-laden water as was found in the overhead cisterns, and the character of food and other factors in influencing the growth of larvæ was studied. The larvæ were collected from other sources and placed in the tanks.

Left in the sun without any cover and the water not renewed

Batch	Tank	Period	Number of days	Food
I	A	25 to 16	29	Dry leaves, grass, dead mosquitoes floating on the surface scrapings from roofs of houses at the bottom
	B	25 to 56	34	
	C	25 to 126	41	
	D	25 to 175	All dead	

Left in the shade from noon onwards

Batch	Tank	Period	Number of days	Food
II	A	6 6 to 17 6	All dead	The nature of food was the same as above, plus moss, decayed leaves. Many flies cut into bits, etc., were dropped in
	B	6 6 to 2 7	27	
	C	15 6 to 17 7	33	
	D	15 6 to 16 7	32	
III	A	18 6 to 12 7	31	Do plus mud from some temporary breeding sites placed at bottom water renewed frequently
	B	2 7 to 28 7	27	
	C	18 7 to 19 7	30	
	D	18 7 to 12 "	25	
IV	A	13 7 to 22 7	10	Food as in Batch III except the depth of water reduced to a few inches and the water renewed very frequently
	B	13 7 to 22 7	11	
	C	20 7 to 29 7	10	
	D	30 7 to 8 8	9	
V	A	24 7 to 2 8	10	Do Do
	B	24 7 to 1 8	9	
	C	30 7 to 7 8	9	
	D	8 8 to 17 8	10	

Thus it is apparent from the foregoing figures that the smaller the depth of water the more rapid is the development of the larva into pupa

NUMERICAL STRENGTH AND MORTALITY

The effect of crowding of larvæ in an overhead cistern was studied by placing a large number of larvæ of the first instar, as determined by their size, in tanks A, B, C and D. Side by side with these observations the rate of mortality was also studied.

Batch	Tank	The number that pupated out of the hundred larvæ added	Mortality per cent
I			
The nature of the food was the same as in Batch I of the experiment mentioned above	A	2	98
	B	2	98
	C	4	96
	D	0	100
II			
The nature of the food was the same as in Batch II of the experiment mentioned above	A	0	100
	B	6	94
	C	8	92

It will thus be observed that the mortality of larvæ was found to be very heavy. It was thought that the available food was not enough for so many larvæ. Hence some mud from a pool, where a very large number of larvæ was found, was added to the tanks and similar observations were continued.

Batch	Tank	The number that pupated out of the hundred larvæ added	Mortality per cent
III			
The nature of the food has just been stated	A	14	86
	B	7	93
	C	15	85
	D	24	76

Even with the increase of food material it was found that larvæ cannot stand overcrowding, at least in permanent breeding places, to any great extent

On the other hand, in marked contrast to what is usually found in overhead cisterns, a small rain water puddle can contain a very large number of larvæ in comparison to the size of the pool and in the latter situation the mortality was found to be much smaller. The observation to determine the rate of mortality in rain water puddles was carried out in a ditch during the rainy season. This was filled up with rain water and in it one hundred larvæ were placed, those that pupated being counted. In this way the mortality in temporary breeding places was found to vary from 57 to 65 per cent

Normally one never comes across any overhead cistern which contains a very large number of mature larvæ at one time, as in course of their development a very large number succumb. On the other hand, in temporary breeding places, as in pools, such increase is very marked, in fact an overcrowding is always noticed

Absence of suitable food is probably the only reason to account for this marked difference in the rate of mortality as observed in temporary and permanent breeding places. Though larvæ will often be found to dive to a depth of say 3 to 5 feet below the surface they cannot make use of the abundant food that may lie at the bottom of this depth of water. On the other hand, the depth of rain water pools is usually a few inches to a foot and thus the larvæ can easily utilize the food to their benefit

EFFECT OF SUNLIGHT ON THE GROWTH OF LARVA

It has been observed that in the vats which are constructed by builders for soaking their bricks and which are often exposed to the sky and to sun's rays throughout the whole day, *subpictus* predominates, provided there is shelter for the larvæ. If there is no shelter probably no larva will be found. On the other hand, there are sites, a portion of which is exposed to the sun only for sometime in the day *stephensi* are more numerous than *subpictus* in these places. The same state of things is observed in ornamental cisterns in the gardens and also in masonry cisterns on the ground for storing filtered water, where larvæ are often found during the rainy season. The best place for finding *stephensi* during the dry season is a collection of water the whole of which is never exposed to the sun all day, only a portion being so exposed, the water thus remains quite cool. During the rains, on the other hand, a large number of larvæ can readily be found in cisterns, vats, rain water puddles, empty tin canisters, etc., which are exposed to the direct rays of the sun

RAINFALL AND HUMIDITY

The two together play a great part in the growth of larvæ and this is apparent from the fact that larvæ thrive well at a time during the rainy season when it is

most humid in other words they grow best at a time when the days are warm and there are frequent drizzles. Such a condition is found during the height of the monsoon and this corresponds with the time when larvæ take on an average from 8 to 10 days to develop into pupæ.

It has further been observed that even in absence of any apparent food material, larvæ grow very rapidly when exposed to the rains. Besides being present in pools and in ditches, larvæ will often be found in wooden troughs, in broken pans, in iron tanks, in broken buckets, etc., during the rainy season. It is difficult to guess the nature of the food larvæ obtain in these places but in spite of the paucity of food they are seen to thrive rapidly.

Small larvæ just emerged from eggs and all apparently of the same age were noticed in two receptacles side by side. One receptacle was removed to the laboratory and the other kept where it was exposed to the rains. It was found that in the former it took 22 days for the first pupa to appear, while in the latter the period was 8 days.

MISCELLANEOUS FACTORS

It has been suggested by Covell (1928) that *A. stephensi* requires fresh water, preferably constantly renewed to breed in and that it will not breed in foul or stagnant water, thus differing conspicuously in its habits from the harmless *A. subpictus*. A careful survey of many of the Calcutta cisterns has revealed the fact that very few larvæ can be obtained from cisterns which are in good order, meaning thereby that the water flows out of these cisterns, whether the latter are provided with lids or not. It is only from unprotected or insufficiently protected and stagnant cisterns that most of our catches have been obtained. Further the relation of food to the growth of larvæ was studied in these cisterns.

Although *stephensi* do not usually occur in foul water, during the monsoon when there is a great tendency for the mosquitoes to propagate their species in a short time, larvæ have on many occasions been found in open earth drains containing foul water and at times in sewage contaminated water, breeding along with *A. subpictus*, *C. fatigans*, *D. obturbans* and probably one or two other species of Culicines.

In connection with the breeding habits of *A. stephensi* of Bombay, Covell has remarked that frequent disturbance of the water, such as is produced, for instance by drawing water from a well by means of a bucket, will not prevent breeding. The writer endorses these remarks as he has had a similar experience in Calcutta. The overhead cisterns, in which these observations have been carried out, were not only unprotected but the water was not flowing. The people were frequently found using this water for washing and for other purposes during the time the filtered water ceased flowing, i.e., from about 10 A.M. to 3 P.M. They were found

refilling the cisterns by keeping the ball in the cistern pressed down till the latter were nearly full. Although the water was thus frequently disturbed, larvæ at all stages could usually be found in them unless there was prolonged overflowing.

CONCLUSION

From the foregoing it will be evident that it is needless to exaggerate the great importance of the influence of external factors on the development of mosquito larvæ. Besides climatic and atmospheric conditions the nature and the character of the food material undoubtedly exert the greatest influence. Although *stephensi* larvæ will often be noticed to dive down to a depth of 4 to 5 feet and as Covell remarks even 20 feet, indeed probably to any depth, they cannot make use of the food that may lie at the bottom unless the depth of water in which the larvæ thrive is a foot or less. They are surface feeders and when the food is present on the surface or very near the surface, they grow rapidly.

The nature of the influence exerted by direct rainfall on larvæ, hastening their growths, even when there is a great paucity of food material, is a matter which requires investigation.

It is likely that *stephensi* is essentially a rainy season mosquito. The temporary pools that make their appearance during this season form the true habitat of this species, while to tide over a critical time like the dry season the nature of the breeding place is completely altered, though this is much less suitable for their growth.

It is quite clear that nature has so arranged that mosquitoes, at least *A. stephensi*, propagate their species at a time not only when the surface for breeding is considerably increased but also when other physical factors favouring their growth are at their optimum, it is then that their numerical strength is at its maximum and the mortality is at its minimum.

The drainage system, also the water works of Calcutta, are recent innovations, say a little over 100 years old. Formerly the source of water for all purposes was from ground wells and every household used to possess at least one. With the extension of modern systems of water supply these wells have been completely closed down. It is not merely a surmise to say that those wells formed the breeding places of *stephensi* and with their gradual disappearance, these mosquitoes have taken to an altogether different habitat.

If it is ever thought necessary that a campaign against *A. stephensi* is to be started in Calcutta, its breeding habits should be first studied on a larger scale before oiling and other measures are adopted indiscriminately. From what has been already said it will be seen that the period of the growth of larva from its emergence from the egg to its metamorphosis into pupa varies greatly not only with the season but also with the nature of the food material present, environment,

temperature, humidity and other factors. A study of the breeding habits will at once show at what intervals in different seasons a particular breeding site has to be attended to with a view to the eradication of this species and this will materially cut down the working expenses.

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ON THE OVULATION OF *A STEPHENSI*.

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[Received for publication, June 8, 1931]

It was thought that one way to determine the preferential feeding habits of one species of Anopheline, a reputed malaria carrier, for the blood of man or any other animal, would be to compare the number of eggs laid after the insects have fed on the blood of man and other animals and then to observe if the progeny are in any way altered in their natural taste for human blood. In this connection other conditions relating to ovulation were studied.

The mosquitoes experimented with were *A stephensi* and were mostly bred out or reared in the laboratory.

In the laboratory the number of eggs laid by a single female after one blood meal, when fed on different animals, was first observed and seasonal variation in their number, if any, was noted as follows —

(The figures in brackets indicate the number of batches of eggs from which the average number has been calculated)

	WHEN FED ON THE BLOOD OF			
	Man	Rabbit	Guinea pig	Rat
<i>Dry season</i>				
Maximum No	95	87	96	101
Minimum No	25	73	59	69
Average No	(36) 49	(5) 83	(5) 77	(7) 85
<i>Rainy season</i>				
Maximum No	94	100	99	104
Minimum No	22	69	71	59
Average No	10, 45	(7) 80	(6) 81	(5) 87

Chalam (1927) observed in connection with the attempted mating of anopheles with culex that though the ovum developed and some mosquitoes even laid eggs after feeding on blood without fertilization, such eggs did not hatch out. Hence it was necessary to find out if the eggs laid by an individual female when fed on the blood of different animals were fertilized or not.

	WHEN FED ON THE BLOOD OF			
	Man	Rabbit	Guinea pig	Rat
Number of eggs laid by a single female	87	75	85	101
Number of eggs hatched out	84	70	83	94

Hence it will be seen that no appreciable difference could be found in the number of eggs laid by a female when fed on the blood of animals such as rabbit, guinea-pig and rat, and the average number of eggs, when the adults were fed on these animals, was found to be almost the same in the two seasons. The average number of eggs, on the other hand, when the adults were fed on the blood of human being, has been observed to be very low. In fact the number was found to be almost half of the other figures. It is difficult to explain this low average in both the seasons unless a large number of experiments are done.

Further, the eggs were all fertile as almost all of them hatched out.

Temperature

The effect of low temperature on ovulation was first observed. It was found that the number of eggs laid by a batch of adults when kept in a temperature of 24°C compared favourably with those laid in the usual atmospheric temperature.

The effect of temperature on the hatching of *stephensi* eggs was next studied. When the eggs were left in water for from 96 to 120 hours in a room having a constant temperature of about 11°C, they were found to retain their viability, as all of them hatched out when left on the laboratory table at ordinary room temperature for about 12 hours, but a similar exposure to the same low temperature for 164 hours was found to kill them altogether.

Desiccation

The resistance of eggs against drying was studied on eggs collected on pieces of wet filter paper, the utmost care being taken not to damage them in any way while picking them up

Nature of desiccation	Eggs hatched out after	1 ggs did not hatch out after
Wet filter paper, remaining moist all the time in a sealed tube, kept on the laboratory table Temperature varying from 80° to 105°F		3 days
do do, kept in a temperature of 11°C	Usually 96 hours Maximum period 120 hours	144 hours
do do, kept in a temperature of 24°C		96 "
Moist clay, left on laboratory table		48 "
Wet cotton-wool and left on laboratory table		48 "
do do, left in a temperature of 11°C	96 hours	144 "
On the side of a porcelain crucible, earthen pot, test tube		3 hours in every case

Chalam (1927), on the other hand, found in Bombay that *stephensi* eggs were viable after 12 days of desiccation when they were left on dried mud from the harbour and dried in a card board box in the room temperature

Mating

The following observations were made in connection with the mating of *stephensi* mosquitoes. In these experiments twelve males and the same number of females were let loose and kept confined for 24 hours. After this period the females were captured and kept in a lamp chimney for 4 to 6 days, they were then dissected and the ovaries were examined to find if they were impregnated

- | | |
|---------------------------|--|
| (1) In a test-tube | 6" × 3", no mating |
| (2) do do | 7" × 1 $\frac{3}{4}$ ", no mating |
| (3) In a glass jar | 3 $\frac{1}{2}$ " × 3 $\frac{1}{2}$ ", 1 impregnated |
| (4) In a cage | (i) 8 $\frac{1}{2}$ " × 6 $\frac{1}{2}$ " × 5 $\frac{1}{2}$ ", 2 impregnated |
| | (ii) 16" × 12" × 8 $\frac{1}{2}$ ", 4 impregnated |
| (5) In a mosquito curtain | 7 $\frac{1}{2}$ ' × 6' × 6', 6 impregnated |

Hence it will be seen that pairing takes place in the air, as the greater the height and the more the space in which mating takes place, the more the number of females that are likely to be impregnated

Progeny of different strains bred out

It was desired to find out the number of eggs that were laid by an individual female, the progeny of adults fed on the blood of different animals. For this purpose males of one strain were left confined with fed females of another strain and the number of eggs laid by the females was noted as follows —

Progeny of a female fed on rabbit's blood—

- | | |
|-----------------------|--|
| (1) ♂ mating with a ♀ | (bred out from cistern)—the number of eggs were 67, 84 and 93 on three occasions |
| (2) ♀ with ♂ | (do do)—72, 70 and 84 |
| (3) ♂ with ♀ | (progeny of a female fed on human blood)—84, 34 and 52 |
| (4) ♀ with ♂ | (do do)—60, 90 and 51 |
| (5) ♂ with a ♀ | (progeny of a female fed on guinea-pig's blood)—55, 85 and 72 |
| (6) ♀ with a ♂ | (do do)—94, 39 and 67 |

Progeny of a female bred out from cistern and fed on rabbit's blood—

- | | |
|------------------------------------|--|
| (7) ♂ with a ♀ | (bred out from ditch collection)—55, 83 and 77 |
| (8) ♀ with a ♂ | (do do)—87, 91 and 90 |
| (9) A large sized ♂ with a dwarf ♀ | —37, 52 and 62 |
| (10) do do ♀ do ♂ | —118, 89 and 98 |

Alteration in the taste of the mosquitoes, the progeny of various strains, for the blood of man and other animals

The eggs in the previous experiments were hatched out and the larvæ reared to imagoes in the laboratory. No striking peculiarity could be detected with regard to their special tendency to feed on the blood of one kind of animal in preference to another. In these experiments man and rabbit were exposed to the bites of these mosquitoes and the number of eggs laid after they were fed on their blood were counted and were found as follows —

- | | |
|--|---|
| Progeny of Nos 3, 4, 5 and 6 Fed on human blood | The number of eggs laid were 54, 74, 69 and 85 respectively |
| Progeny of Nos 3, 4, 5 and 6 Fed on rabbit's blood | The number of eggs laid were 73, 81, 39 and 49 respectively |

Number of eggs in proportion to the size of the insect

When a large number of *stephensi* are bred out from a collection of larvæ from some ditch, the adults will usually be found in three different sizes. One is extraordinarily large, the other medium and the third is undersized or dwarf, and the

number of eggs laid by them on three different times were as follows—all having been fed on man —

Stout	118 95 and 100
Medium	75, 101 and 87
Dwarf	31, 78 and 43

Hence it will be seen that the number of eggs laid by a mosquito is in proportion to the size of the mosquito

Relation of ovulation to blood feed

A blood feed has been found to be absolutely essential for the ovum to be fertilized. In case where the blood feed has been delayed and sufficient time has elapsed after the mating, the mosquito will lay eggs usually on the second or the third day after it has had a blood meal. An interval usually of from six to eight days is necessary before the mosquito can lay eggs for the second time and again an interval of from 7 to 10 days before the third batch of eggs is laid. The number of eggs laid in different batches is shown below —

Mosquito	Batch 1	Batch 2	Batch 3
A	(fed on man) 85	(fed on man) 69	(fed on man) 12
B	(fed on rabbit) 85	(fed on man) 95	(fed on man) 33
C	(fed on rat) 83	(fed on man) 34	(fed on man) 42
D	(fed on rat) 101	(fed on man) 49	(fed on man) 23

The mosquito E, on the other hand, was found to behave in an altogether different way. The number of eggs on different occasions and the interval between successive batches of eggs is shown below —

Fed on rabbit	85 eggs laid on 25-4-31
Fed on man	95 " " " 30-4-31.
do do	47 " " " 3-5-31
do do	42 " " " 8-5-31
do do	41 " " " 11-5-31
do do	45 " " " 14-5-31.
do do	48 " " " 16-5-31
do do	37 " " " 21-5-31
Fed on raisins	

No further batch of eggs was laid and the mosquito died on 26-5-31

The writer's observations, as stated above, are not in accord with those of Christophers, Sinton and Covell (1928) who report on the period taken for development of second and subsequent batches of eggs as follows —

‘Immediately after laying a batch of eggs the oncoming follicles are as we have seen, already well advanced in development and subsequent batches of eggs follow at a shorter interval. Immediately after oviposition the second follicle is in a stage representing about 48 hours' development. The second batch of eggs, therefore, instead of taking 6 days to develop, under the conditions noted, took only some 4 days to develop. That they may develop in an even shorter time is suggested by the state of affairs sometimes found in *Anopheles* in nature.’

When the female has been fed on vegetable juice alone, e.g., raisins, during the interval, it has not been possible to induce the mosquitoes to lay any further eggs after a batch of them has been laid. So far it has been found in the laboratory that they need a blood meal during the interval of successive batches of eggs.

Sometimes one batch of eggs is laid in two instalments on two successive days and on rare occasions in three instalments on three successive days.

CONCLUSION

All these observations, mentioned above, tend to show that *Calcutta stephensi* will readily feed on any warm-blooded animal and after a blood meal will lay fertile eggs, this points to the catholicity of the feeding habits of this mosquito. The mosquitoes of the various strains reared in the laboratory did not seem to be in any way restricted in the choice of their hosts.

In connection with the mating it was observed that space has a direct bearing on pairing as the greater the space and the height, the more the number of females that are impregnated as the mating takes place in the air.

It has not been possible for us to keep the mosquitoes alive sufficiently long to get more than three batches of eggs, though one lived for a little over 5 weeks and produced 8 batches of eggs, this being considered an exception rather than the rule. In a subsequent paper the writer will deal with the relation of the longevity of the various strains to atmospheric temperature and humidity.

It has been shown that a blood meal is essential not only for the fertilization of the first batch of eggs, but also for each successive batch.

With regard to the desiccation of *stephensi* eggs, experiments point to the fact that they are very delicate and cannot stand drying in any way except for a very short period.

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ON THE BREEDING HABITS OF *A STEPHENSI* AS OBSERVED IN THE LABORATORY

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THE essential object of this work was to find out the best way to breed one of the commonest species of Anophelines, susceptible to malaria, and to keep the culture going in the laboratory, thereby the difficulty of obtaining a large number of such mosquitoes for experimental purposes being obviated

No difficulty was experienced in inducing the mosquitoes to oviposit in the laboratory and it was noticed that most of the eggs hatched out. The difficulty encountered was in rearing the larvæ. At first it was thought that success would be ensured if they were reared exactly in the same way as found in nature and so the small larvæ after their emergence from eggs were dropped in non cisterns filled with the unfiltered water of Calcutta. But it was soon found that only a small number matured into pupæ, while the majority of them succumbed during the course of growth, and as the success obtained was so little, this method of rearing the larvæ in the laboratory had to be abandoned.

The next attempt to rear them was in circular earthen pots with a diameter of, say, a foot in which some mud and other organic substances were placed at the bottom. It was considered that these substances would be suitable to provide them with their food. But this method too was found to be unsatisfactory, as a very large number of small larvæ disappeared, presumably due to uncongenial surroundings. Similar pots with deposits of green algæ at the sides were next used and these were found to be no improvement over the former. After a large number of similar trials the following was found to be the best way of rearing *A stephensi* in the laboratory.

A rabbit enclosed in a cage was kept inside a large mosquito curtain for the night, in which a large number of mosquitoes, both males and females, were let

loose Long hollow tin canisters were placed on the floor inside the curtain for shelter of the mosquitoes. The cage with the rabbit was taken out in the morning when the tray was cleansed and the food was changed, the mosquitoes not being disturbed at all. The pots were removed when the eggs deposited in them hatched out and a fresh pot was placed inside. Small larvæ were then carefully picked up by means of a pipette and kept in a small earthen pot containing plenty of ordinary grass, the ends of the blades projecting out of the surface of the water. Thus the portion of the grass projecting out of the surface remained alive for a considerable time and thus helped a great deal in the oxygenation of the water. The grass serves the following useful purpose: first, it keeps the water cool, secondly, it separates the water into different compartments and thus the larvæ are prevented from preying on one another, thirdly, it itself provides some food for the larvæ, and fourthly, it prevents the formation of the scum so frequently noticed on the surface of water and which quickly asphyxiates the larvæ.

When earthen 'gumlas' are not available equally good results will be obtained with ordinary enamelled pans with raised edges.

The best food which has been found to ensure rapid growth of larvæ is finely powdered manure lightly sprinkled over the surface of water, and also dead mosquitoes filled with blood. Even ordinary dust will serve the same purpose. It must be remembered that *Anopheles* larvæ are surface feeders and *A. stephensi* is no exception, hence whatever food is given, it must be available to the larvæ on the surface.

Even in the apparent absence of much food larvæ will often be found to thrive well when they are left in the shade in earthen pots exposed to the rains. Drizzles, i.e., when the rain falls in minute drops, have been found to stimulate the growth of larvæ much better than rain falling in larger drops. Larvæ are not likely to get much food in these earthen pots or 'gumlas' except a few dead insects and some green algæ which rapidly grow on the inside of these pots when they are left in the shade during the rainy season. When larvæ in two 'gumlas' are compared, one with scanty growth of green algæ on the sides without any other food material and exposed to the rains and the other having abundant food material but left in the shade and not exposed to the rains, it will be found that the larvæ in the former will always grow much more rapidly than those in the latter.

It has been found after repeated experiments that the depth of the water in the pot in which the larvæ are reared is important and care should always be taken that too much water is never poured in these breeding pots.

Why it should be necessary to change the water of the pots in all breeding experiments in the laboratory, even when they are kept out all the time in any places, is difficult to say. The explanation that defective oxygenation or increase in CO_2 necessitates the constant changing of water is not sufficient for the reason

that in nature larvæ are sometimes found in places such as wooden troughs, tin canisters, etc., during the dry season and the water remains stagnant all the time. But any way one should always pay special attention to the changing of the water very frequently, say every 3 to 4 days, even when no scum forms at the surface.

While rearing Anopheline larvæ in the laboratory it will be noticed that a very large proportion of small larvæ will always succumb. The writer has already pointed out elsewhere the proportion of larvæ which die in their development from the smallest stage to pupation under different conditions in nature. In the laboratory, on the other hand, the mortality of larvæ has been found to vary greatly, ranging from 97 to 60 per cent, when the conditions have been made most favourable to their growth. During the course of metamorphosis the mortality has been found to be heaviest during the first and next heaviest in the second stage while the mortality is the lowest in the last larval stage. It is difficult, and at the same time trying, to rear in the laboratory larvæ of the first instars.

It can be stated from observations in the laboratory that the mortality of larvæ depends, among others, on the depth of water in which the larvæ grow, presence of suitable food material easily available to them at the surface and on the number of larvæ.

The greater the depth of water, however abundant food materials there may be at the surface, the more the number of larvæ that will die. Given two pots of different depths of water, one shallow, i.e., the depth of water not more than a few inches and the other deep, i.e., the depth of water from $1\frac{1}{2}$ to 2 feet, the nature of the food being the same in both, it will always be found that larvæ will grow into maturity more quickly in the former than in the latter. Also the mortality in the former situation will be much less than in the latter, where the water is deep.

It is needless to repeat that Anopheles mosquitoes are surface feeders and hence in absence of food at the surface a very large number will readily perish.

It has already been mentioned that in permanent breeding places of *A. stephensi* a given surface of water can maintain a limited number of larvæ only and when the number exceeds that limit the rest will die, while in temporary breeding places an area of the same dimension can contain a very large number of larvæ. In these experiments under the natural conditions it was not possible to determine the fate of those that disappeared. With a view to ascertain this, experiments were done in shallow enamelled dishes in which a large number of larvæ of all sizes, previously counted, had been dropped. They were then counted from day to day and it was found that almost all the small larvæ had disappeared in course of 3 to 4 days, only a small number among them surviving. These were thought to have been preyed upon by the larger ones. Evidence pointing to this was found in the absence of any trace of the bodies of the small ones, and in the large number of heads of bigger larvæ left at the bottom, while the bodies of the dead ones, lying at the bottom were very few. It will not be out of place to point out that although

at times larvæ are carnivorous they do not touch dead or decayed larvæ. One who has taken the patience to watch a pot containing a large number of larvæ of all sizes will find no difficulty in observing the carnivorous habit of *Anopheles* larvæ. This will be dealt with more fully in a subsequent paper.

The best way, I have found, whereby one can materially reduce the mortality is not to touch young larvæ at all for three to four days after their emergence from eggs but to let them grow in the same pot where the eggs were laid. Larvæ at this stage do not require much food but some fine dust may be very lightly sprinkled twice a day on the surface. After 4 days when they are large enough to handle, they can be picked up by a pipette and transferred into an enamelled dish, or better still, an earthen 'gumla,' with plenty of grass with roots in it, the water being changed every three to four days along with the grass. Powdered manure or dust should be lightly sprinkled on the surface twice every day. The breeding pots should be put out in a well lighted place.

The maximum and the minimum time taken by larvæ to pupate under natural conditions has already been stated. In ordinary surroundings in the laboratory the maximum period has been found to be as long as 58 days (if similar results had been obtained during the winter season this unusual delay would probably have been explained as 'hibernation'), while the minimum time for larvæ to pupate has been found to be 9 days. But these are quite unusual cases. It is indeed impossible to say with any degree of accuracy the length of time larvæ will take to pupate in the laboratory. The conditions on which this depends are too many and very little known. While one batch takes, say, 12 days, another batch under the same conditions may take twice that number of days or even more.

The effect of low temperature on the development of larvæ has been studied. When subjected to a constant temperature of 24°C, they usually take about 16 to 20 days to pupate, while in a temperature of 12°C the large ones do not survive for more than 3 to 4 days and the small ones for more than 24 hours. At this low temperature they do not feed and the larvæ do not wriggle about, but when brought into warm surroundings they soon become active and begin to feed.

The writer cannot explain the reason why a batch of adult mosquitoes in the course of breeding stopped laying any further eggs almost abruptly. This happened when the breeding operations were going on in full swing. The adults referred to were all bred out in the laboratory and were probably more than three broods old. Although the females were noticed to have fed on rabbit's blood they could not be induced to lay eggs even outside the curtain, which was adapted as a breeding cage. For this purpose some fed females together with males were kept confined in cages and also in lamp chimneys but with every effort the mosquitoes could not be induced to lay any eggs. Some of the females which had refused to lay eggs were then dissected and the ovaries were found in the same stage of development.

as when bred out. The whole operation thus had to be started fresh from the very beginning. The writer has since then been informed that an entomologist had had exactly similar experience while working with an altogether different species of insect. In his case also the cycle of life stopped abruptly at the adult stage. Hence it is advisable that adults, bred out from larvæ caught in nature, should always be introduced inside the breeding cages from time to time so that a continuous supply of adults in the laboratory may be obtained.

The difference, if any, in the rate of maturation of larvæ and then pupation, when unfiltered silt-laden water, ordinary tap water and water from some tank is used, has been found to be quite negligible.

AGGLUTINATION IN LEISHMANIASIS *

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[Received for publication, June 11, 1931]

SEROLOGICAL observations in Leishmanial diseases, though by no means scanty, are somewhat conflicting as will be seen from the accompanying summaries. The difficulties connected with the culture technique in obtaining adequate quantities of a suitable antigen may account to a certain extent, for the want of uniformity in the results recorded, to this may be added that in protozoal infections in general the reaction products are not so abundant or so persistent and the different anti-bodies are not so easily demonstrable as in bacterial infections—a feature one would naturally expect in view of the grade of heterogeneity and the differences in the constitution of the antigens derived from two such biologically distinct parasites.

Now that it is possible to obtain a pure antigen in bulk and of requisite potency by cultures of *Leishmania* on solid media(31), an attempt at a reinvestigation of the subject of agglutination had been made in this piece of work.

From the summaries above referred to, it will be seen that in spite of the conflicting results obtained by several workers, serological tests, e g, the complement fixation and agglutination have enabled some observers not only to differentiate *Leishmania* as a group from other flagellate infections but also to establish even the diagnosis as to the identity of the different members of the Leishmanial group of parasites.

When one, however, comes to examine the properties of the antigen now available in bulk, and the vast biochemical differences in the potency of different

* I desire to express my thanks to Dr L E Napier, Dr C G Pandit, Major Amboj Bose, I M S, and Dr J D Warma for their kindness in supplying the sera and also to my friend Dr P V Gharpure of this Institute for his constant help in this investigation.

anti-bodies demonstrable in the sera of individuals infected, either by live virus, or its dead products as the case may be, in generalized or localized disease and in natural and experimental Leishmaniasis, and also when one finds the preponderance of group anti-bodies over specific anti-bodies in all these several circumstances, it may be pardonable, if one hesitates, to accept the findings of the previous investigators as final. This is particularly in reference to the identification of the several members of the class, based on the quantitative serological reaction and that too within such a narrow range.

Properties of antigen prepared by several methods and their bearing on the choice of a standard antigen

Leaving aside the antigen prepared from the watery or saline extract from the spleen rich in parasites, or from the flagellates centrifuged from cultures in different fluid media whose characters would be obviously not uniform, attention has been concentrated in the study of the properties of the antigen prepared from known quantities of surface growths of a certain age on solidified media of a definite composition.

The chief value of the *Leishmania* cultures grown on the surface of solidified media in contrast with the flagellates gathered from the centrifuged and washed deposits from cultures in liquid media, lies in the fact that apart from the purity and richness of the material, the suspension made from the former can be obtained free from the rosettes and auto-agglutinated masses characteristic of the latter, and that is why antigen made from the surface growths is admirably suited for agglutination tests. An ideal fluid for this purpose then would be a flagellate suspension which would be free from such self-formed clumps to begin with, and such as would not only not tend to their spontaneous formation when left to itself or when mixed with neutral sera used for control but also such as would remain so, for at least six hours, the strictly restricted time allowed for observation beyond which the suspension would, owing to the weight of some of the flagellates tend to deposit by itself however slightly, and lead to fallacious readings especially in doubtful cases.

Characters of suspensions in distilled water and their drawbacks

Such a suspension is obtained by taking up the surface growth of four days at 22°C in distilled water. In this the flagellates swell up and plasmolyse yielding a colloidal solution of the cytoplasm, in which the nuclear and flagellar matter is found suspended uniformly in a finely granular condition. But unfortunately such a suspension is unfit for the test as the colloidal solution becomes turbid and precipitates in contact with even normal saline.

Importance of using a vehicle with normal saline tonicity

Therefore it is essential to have a vehicle, for the suspension, an isotonic saline solution, or better still a hypertonic saline which can be reduced to the normal by adequate addition of distilled water just at the moment of mixing

Drawbacks of using suspensions of living flagellates

A young surface growth, made up in saline with a certain amount of agitation, is found to consist of individual and discrete flagellates actively moving about in H D preparation, but these soon become immobilized in masses at the room temperature (32°C) and tend to agglutinate in a few minutes when mixed with neutral control sera even in 1 in 30 dilution with normal saline. This defect is obviated by killing the flagellates at 55.5°C for one hour or by exposing them to the vapour of CHCl_3 ,

The value of using suspensions of flagellates killed by heat or CHCl_3 vapour

The microscopic examination of these suspensions both in H drop or in stained smears show the discrete distribution of morphologically unaltered flagellates and even a three or four hours' contact with neutral control sera (1 in 30 dilution) are inoperative in producing the clumping of the flagellates which are very sensitive when alive

The consideration of the property of aqueous or saline suspension of flagellates alive or dead have lead the author to adopt the following technique for obtaining the standard antigen used for the agglutination phenomenon

TECHNIQUE

A four days' culture of *Leishmania* grown on the surface of the solidified hæmoglobin(31) at 22°C is gathered on a loop and shaken up in 2 c c of NaCl, 0.85 per cent and when the suspension is quite homogeneous and uniform, it is submitted to 55.5°C for one hour and then left at 22°C for twenty-four hours or more so as to let the heavier flagellates settle down. The supernatant part of the suspension is then carefully sucked up in a pipette and transferred to another test tube and is then found to be of uniform opalescence corresponding to Brown opacity No. 2 which remains so for over twelve hours—and when examined microscopically is found to consist of discrete individuals of dead flagellates unaltered morphologically and free from rosettes or auto-agglutination masses. This is the standard antigen used and it keeps for over a month, although at the end of twenty-four hours a fine deposit is noticeable at the bottom

The most convenient serum dilutions and the time limit for observations

After a great many trials it was found that a dilution of 1 in 30 is the most convenient for the routine examination of sera as with this dilution the controls remain unaltered even up to eight hours, after which time there is a tendency for the heavier flagellates to settle down. In all K A sera, the agglutination is obvious to the naked eye in two hours and complete in four hours, however, in some cases a positive reaction is demonstrable even in 1 in 120 dilution at the end of six hours. Therefore a definite time limit (of three hours) has been fixed for recording the readings for 1 in 30 dilutions.

THE TEST

An equal quantity of diluted serum and antigen suspension is taken up in sealed capillary pipettes with an approximately uniform bore of about 1 mm and left after sealing in a vertical position at the laboratory temperature, always having a control serum mixture for each group of sera tested and at the end of two hours an examination with a lens reveals the formation of granules, which when the reaction is positive grow larger and become quite visible to the naked eye by three hours and when the test is complete, these deposit like a mass of clot leaving a clear transparent supernatant column of the fluid, while the contents of the control pipette remain unaltered with its homogeneous and uniform opalescence. The phenomenon can be illustrated in permanent preparations by sucking up the contents of the tests at the end of three hours and filming this on slides—fixing and staining the films—*vide* Plate XXXVII.

LIMITATION OF THE SPECIFICITY OF THE REACTION

A large number of observations made on gross agglutination of the L D and L Tr suspensions by oriental sore or kala-azar sera and vice versa did not reveal any distinctive features thus showing that it is immaterial from what particular source of *Leishmania* the suspensions are derived. The observations made indicate the preponderance of group agglutinins over specific agglutinins when they are present. It is found that suspensions with L Tr cultures can be used with equal effect in all agglutination tests whether the serum is derived from cases of kala-azar or oriental sore. In this work on sera derived from only kala-azar and oriental sores (natural and experimental) from different parts of India, L Tr antigen has been mainly adhered to for demonstrating the presence or absence of agglutinins. The results obtained from these variants seem sufficiently clear to indicate the presumption that the agglutination phenomenon being as it is only a group reaction may be extended with equal effect when dealing with *Leishmania* from other parts of the world. Sera from other *Leishmanial* infections appears to be of little value in the diagnosis of the different members of the parasites.

PLATE XXXVII

AGGLUTINATION—L Tr Suspension as Antigen

Control Serum 1 in 30

K A Serum 1 in 30

O S Serum 1 in 30

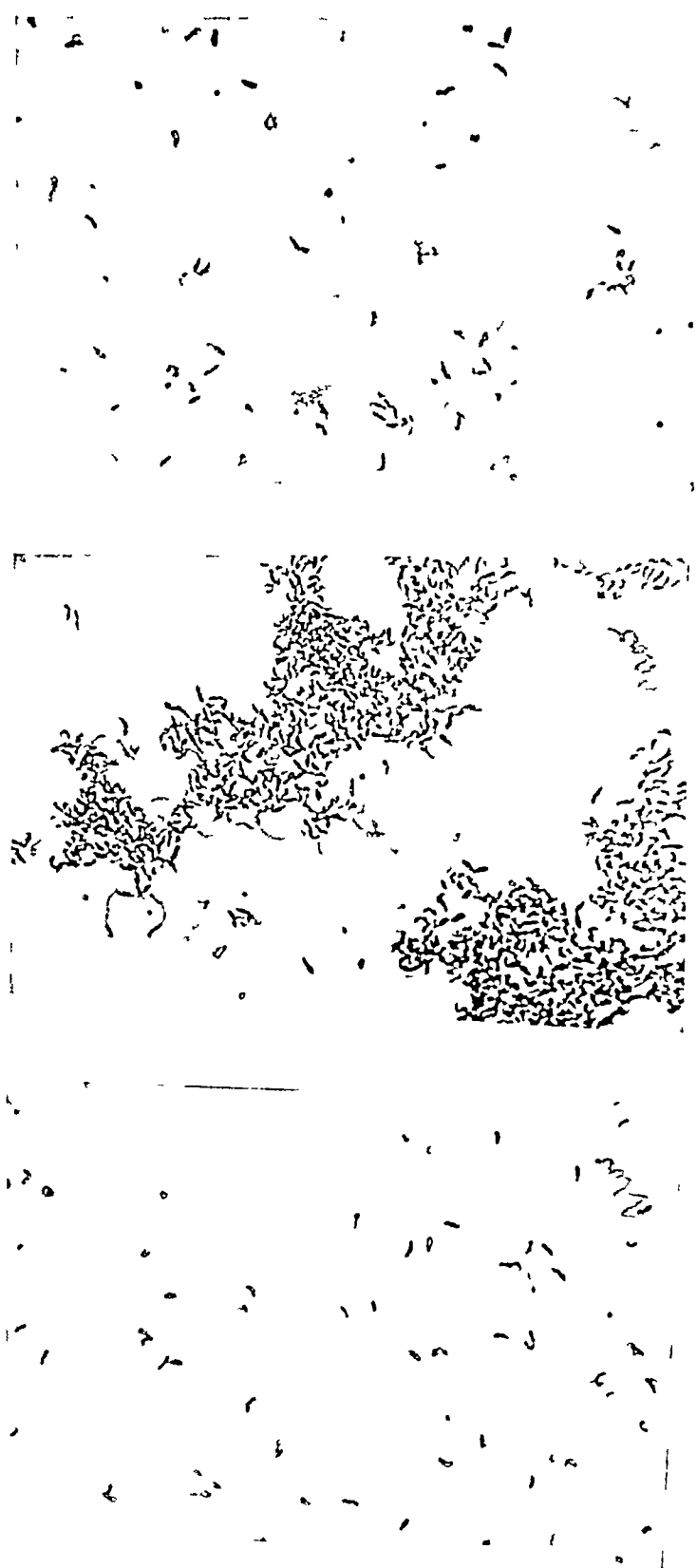


Fig 1
(Human)
Expt X

Fig 2.
Case 6, Madras Series
Expt V

Fig 3
Case 8, Lahore Series
Expt XV

PHOTOMICRO—Ocular X and Objective C Zeiss

(Abbreviations—L Tr=*Leishmania tropica*, K A=Kula azar, O S=Oriental sore)

The results of these experiments are summarized in the following tables —

TABLE I

Agglutination in kala-azar serum, Calcutta Series

Serial No	Serum nature and source	Clinical data	Aldehyde test of Napier	Serum globulin test of Brahmachari	Treatment	Agglutination serum 1 in 30 antigen L Tr L D	
1	Calcutta K A advanced	Clinically diagnosed as K A by Napier	+	+		—	
2	” ”		+	+		—	
3	” ”		+	Capsule found broken in transit			
4	” ”		+	Capsule found broken in transit			
5	” ”		+	+	All untreated	++	
6	” ”		+	+		++	
7	” ”		+	+		++	
8	” ”		+	+		++	
9	” ”		+	+		++	
10	” ”		+	+		++	
11	” ”		+	+		++	
12	” ”		+	+		++	
13	” early		+	+		++	
14	” ”		+	+		++	
15	” ”		+	Capsule found broken in transit			
16	” ”		+	+			++
	Old serum ? Napier		+		++		
	Control		0	0	0		

Abbreviations used in the tables

K A — Kala azar serum
 L Tr — *Leishmania tropica*
 ++ Agglutination distinct in 2 hours, complete in 3 hours
 + " " " 3 " " " 4 "
 + ? " " " 6 " but incomplete in 6 hours
 0 " negative

O S — Oriental sore serum

L D — *Leishmania donovani*

TABLE II

Agglutination in kala-azar serum, Madras Series

Serial No	Serum nature and source	Clinical data	Aldehyde test	Treatment by urea stibamine	Agglutination serum 1 in 30 antigen			
					L	Tr	L	D
1	Kond Mad K A	Spleen — Liver — Fever 8 days	+	0	+			
2	K K 22 „ „	Spleen — Fever 3 months	0	0	+			
3	D M 22 „ „	Spleen — — Liver — — Fever 2 months	0	0	+			
	Control			0	0			
4	L R Mad K A	Spleen — Fever 3 months Cancerum Oris		0	++			
	Control				0			
5	W I Mad ?	Spleen — ? No history	0	0	0			
6	D M Mad K A	Spleen — Fever one week General condition poor	+	0	++			
7	N C „ „ „	Spleen — ?	+ ?	0	++			
8	K 30 „ „ „	Spleen — ? No history of fever	+ ?	12	+ ?			
	Control		0	0	0			
9	Rajamma K A Mad	Liver — Spleen —	+	0	++			
10	Natesan Mad K A	Spleen — Liver — Fever 4 months	+	0	++			
	Control		0	0	0			

TABLE III

Agglutination in kala-azar serum, Patna Series

Serial No	Serum nature and source	Clinical data	Aldehyde test	Treatment Neostibosan injections	Agglutination serum 1 in 30 antigen			
					L	Tr	L	D
1	S R P Patna K A	Spleen ++	+	0	++			
2	W A " " "	Spleen ++	+	2	+			
	Control			0	0			
3	Mos Patna " "	Spleen ++	+	1	+		+	+
4	L N " " "	Spleen +	+	8	+	+	0	
	Control	Spleen—Malaria	0	0	0			
5	B Patna K A	Spleen --	+	1	++			
6	R S Patna K A	Spleen --	+	1	+			
7	G S " "	Spleen —	+	1	0			
8	A R " "	Spleen —	+	12	+			
9	R Patna K A	Spleen --	+	5	+			
10	Ampoule broken in transit							
11	D Patna K A	Spleen --	+	1	++			
12	R " "	Spleen --	+	2	++			
13	B " "	Spleen --	+	2	+			
	Calcutta K A		+		+			
	Control	Spleen 0	0	0	0			
14	R L Patna K A ?	Spleen —	0	0	0			
15	S Patna K A	Spleen —	+	5	+			
16	D M Patna K A	Spleen —	+	8	++			
17	Ja Patna K A	Spleen —	+	0	++			
	Control	0	0		0			

TABLE IV

Serum reaction (agglutination) in oriental sore, Lahore Series

Serial No	Serum nature and source	Clinical notes	Agglutination serum 1 in 30 antigen		REMARKS
			L	Tr L D	
1	O S Lahore	Nothing available	0		Agglutinins though absent in O S sera are easily produced by the rabbit in response to injections of the products of <i>Leishmania tropica</i> or <i>Leishmania donovani</i>
2	" "	" "	0		
3	" "	" "	0		
4	" "	" "	0		
5	" "	One ulcer, 2 months	0		
		One nodule, 3 weeks	0		
6	" "	Ulcer, 1 month	0		
7	" "	Ulcer angle of mouth, 3 months	0		
8	" "	Nodular forearm, 1 month	+	?	
16	K A Patna		+	+	
	Control		0		
	Rabbit immunized to L Tr—2 injections (2 weeks' interval) of culture		+	+	+
	Control rabbit		0		
9	O S Lahore	Ulcer cheek, 3 months	0		
10	" "	Ulcer forearm, 6 months	0		
11	" "	Multiple ulcers face and arm, 2 months	0		
12	" "	} Not available	0		
13	" "		0		
14	" "	Nodular non ulcerating, 4 months	0		
15	" "	Ulcer leg, 12 months	0		
16	" "		0		
	Control human serum for each batch		0		
	Ghate—Persia O S, 6 months' history deep ulcers L Tr found in lesions		0		Before treatment by L Tr vaccine
	Sonbai—Bombay 1 year's history, multiple sores, diagnosed microscopically L Tr found		0		

From the above it is clear that—

1 The agglutination is a group reaction

2 Agglutinins are definitely demonstrable in all the cases of kala-azar, in some more than in others, the former being always in untreated cases and the latter in most of the cases treated with some form of antimony salts. The only exceptions to the rule are No 14 (Patna Series) where the aldehyde test being also negative there is a certain amount of doubt as to the diagnosis, and No 5 from (Madras Series)

3 The agglutinins are conspicuous by their absence in cutaneous leishmaniasis the only exception being No 8 of (Lahore Series) where there was a feeble effort at agglutinin production

4 These anti-bodies are, however, most easily elaborated in the rabbit in response to the injection of the products of *Leishmania*

5 The agglutination test being an antigen anti-body reaction is of scientific interest. I do not hold that it will replace for practical purposes of clinical diagnosis, tests such as the aldehyde test of Napier, serum globulin reaction of Brahmachari, and urea stibamine reaction or flocculation test described by Chopra

6 The agglutination tests are in conformity with the author's findings as to the close affinity of L Tr and L D evidenced by the identical processes induced experimentally by either virus in the monkey and in the mouse, viz, a cutaneous localized nodule being produced in the monkey with L D and generalized infection induced in the mouse by L Tr (8, 10, 12)

7 The agglutination test is of no value in arriving at a differential diagnosis of the several members of *Leishmania*

SUMMARIES OF THE FINDINGS OF PREVIOUS WORKERS *

SUMMARY 1

Agglutination in leishmaniasis

<i>Date</i>	<i>Investigators</i>	<i>Findings</i>	<i>Antigen used</i>
1909 10	Nicolle and Manceaux	Dog infected with <i>L. donovani</i> No agglutination	15 days culture of L D NNN
1911	Jemma and di Cristina	Infected infant No agglutination	Aqueous and alcoholic ext of K A spleen
1912	Longo		Saline ext of dried splenic pulp

* The author is indebted to W H Taliaferro, from whose excellent work on 'The Immunity of Parasitic Infection' these summaries have been mostly compiled

SUMMARY 1—*concl'd*

<i>Date</i>	<i>Investigators</i>	<i>Findings</i>	<i>Antigen used</i>
1911(6)	di Cristina	Artificially immunized animals by L D Specific agglutination positive	L D cultures in NNN
1912	Caronia	Child immunized by killed culture Positive agglutination	do
1913	Caronia	Natural infantile leishmaniasis four out of five positive agglutination Eight other negative, but these gave positive agglutination after immunizing with killed culture or the nucleoprotein products	Saline ext of dried splenic pulp
1913	Caronia and di Cristina	Two healthy children and two infected After immunization positive agglutination	do
1914	Scordo	Human cases—agglutination pore potent Canine cases—agglutination less potent	Culture of L D human
1914	Archibald	In K A patient No agglutination	Live culture (washed flagellated)
1916	Cornwall and La Frenais		
1918	Oslen		
1927	Aurecchio		
1926	Hindle, Hou and Patton	On immobilization in human and animal serum Results inconstant	Live culture of L D

SUMMARY 2

Complement fixation in leishmaniasis

<i>Date</i>	<i>Investigators</i>	<i>Findings</i>	<i>Antigen used</i>
1911	Jemma and di Cristina	Early work—negative	Aqueous and alcoholic ext of K A spleen
1911	Makhas and Papassati-riou	<div style="display: flex; align-items: center;"> <div style="margin-right: 10px;"> 5 cases of K A 3 syphilis 2 malaria 2 healthy </div> <div style="font-size: 3em; margin-right: 10px;">}</div> <div> All negative </div> </div>	Aqueous ext of K A spleen (Infantum)
1911	di Cristina	<div style="display: flex; align-items: center;"> <div style="margin-right: 10px;"> 3 rabbits immunized positive 3 rabbits fresh negative </div> <div style="font-size: 3em; margin-right: 10px;">}</div> </div>	Saline ext of dried splenic pulp

SUMMARY 2—concl'd

<i>Date</i>	<i>Investigators</i>	<i>Findings</i>	<i>Antigen used</i>
1912		7 human infected with K A — negative	Saline ext of dried splenic pulp
1912	Longo	3 children infected K A — negative	do
1912	di Cristina and Caronia	7 infected human but immunized with killed K A cultures—positive	do
1912(b)	di Cristina	2 cases of spontaneous recovery positive	
1913	Caronia	1 case infected but immunized with K A culture—positive	
		8 cases which gave negative after immunization gave positive	
1913	Caronia and di Cristina	Only 13 out of 88 cases (partial or definitely positive)	Alcoholic ext of spleen from canine leishmaniasis
1914	Pavoni	2 cases of O S positive 1 K A case negative K A cases recovered positive	Aqueous ext of infected spleen and K A infection
1916	Cornwall and La Jrenais	1 case of K A negative	Alcoholic and saline ext of spleen
1917	Brahmachari	6 out of 8 gave positive	Alcoholic and saline ext of spleen
1918	Oslen	2 cases of K A negative	
1920	Knowles	4 cases of K A negative	
1925	Kasuga and Tamura	Immunized animals—certain degree of fixation	
1926	Hindle, Hou and Patton	19 out of 25 well marked fixation	Saline ext of hamster's spleen, glycerin and phenol
1927	Auricchio	21 out of 24 positive 3 out of 24 negative	Flagellates from cultures
1914	Pavoni	One case of dermal leishmaniasis positive 2 cases of O S positive Homologous K A sera negative	Aqueous ext of K A spleen
1919	Moses	80 per cent in 41 cases positive	Culture of flagellates aqueous ext

SUMMARY 3

Serological tests for differential diagnosis of leishmaniasis

<i>Date</i>	<i>Investigators</i>	<i>Findings</i>	<i>Antigen used</i>
1910	C Nicolle and Manceaux	L T protected against O S L I slightly against K A L D against both	Group reaction
1911	di Cristina	Rabbit immunized by L D showed agglutination in 1 in 30	Aqueous and alcoholic ext of infected spleen
1913	Bandi	Established identity of L D <i>L infantum</i> by agglutination of 1 in 160 and non agglutination of L T of 1 in 70	
1913	Caronia and di Cristina	Confirmed Bandi as to identity of L D, L Inf, and L Camis by cross agglutination	Aqueous ext of dried K A splenic pulp
1912	Row, R	Evidence of close affinity between L D and L Tr from path process induced experimentally in the monkey and the mouse L D giving rise to local lesion in the monkey L Tr inducing generalized leishmaniasis in the mouse	Experimental infection with the virus in animals
1913			
1914			
1914	Pavoni	Cross complement fixation experim K A and natural O S sera gave positive agglutination	Aqueous ext of spleen
1914	Scordo Spagnolio Guigni	K A serum gave stronger agglutination with human virus than with canine virus	
1917	Laveran	Could not confirm this Monkey cured of O S gave positive infection with L D Monkey immunized to K A by L D and L Inf gave positive infection with L T	Cross immunity experiment
1924 and 1926	Noguchi	Obtained clear cut results by immunizing rabbit to L D, L Inf, L Tr, and L Brazil, and by cross agglutination test was able to separate all <i>Leishmania</i> into 3 classes including L Br, L D, L Tr and L Brazil	Immunization of rabbit and cross agglutination and living culture

SUMMARY 3—concl'd

Date	Investigators	Findings	Antigen used
1926	Kligler	All findings confirmed	
1926	Wagner and Koch	Separated <i>Leishmania</i> from <i>Herpetomonas clenoccephali</i> Also group reactions in <i>Leishmania</i> not appreciably different	
1926	Noguchi	Separated 3 species of <i>Leishmania</i> <i>Herpetomonas</i> of plants and insects by immu- nity experiment in rabbit	Agglutination with various flagellates
1928	Burowa	Separated L D from L Tr by experimental <i>Leishmania</i> in mice	Live cultures
1928	Chodukine and Soffieff	By immunization of mice guinea pigs and rabbits with different strains was unable to differentiate L T and L D and L Canis by adhesive phenomena	Live cultures
1930	Zdrodowski and Voskeressenski	Differentiation between K A and O S by complement fixation	Killed cultures plus glycerin 1 per cent, phenol 0.5 per cent
1931	Zdrodowski and Voskeressenski	Differential diagnosis of L D, L Tr, L Canis, L Inf and L Brazil by complement fixation	

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RAT-FLEA SURVEY OF THE CITY OF HYDERABAD (DECCAN).

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[Received for publication, June 16, 1931]

HYDERABAD, the fourth largest city in India, is the capital of H E H the Nizam's Dominion. The municipal limits of the city include an area of 35 square miles and with the suburbs quite close to it the area covered is nearly 60 square miles. It is situated 17°22'N Lat, and 78°27'E Long. It is 1,719 feet above sea level.

Population —The population according to the census of 1931 is 375,000 (provisional figure)

Housing and sanitation —The river Musi, a tributary of the river Krishna, divides the city into two parts, that on the southern side of the river being known as the City proper and the one on the northern side being known as Chaderghat. The former is quite typical of an oriental city and consists of several palaces surrounded by areas with narrow lanes lined on either side with small, ill-ventilated, 'kucha' houses with country tiled roofs. Chaderghat comprises a wider area and includes recent extensions. Several small, once independent, villages have become included. This part, particularly in the localities near the grain markets, is badly congested, the houses are mostly 'kucha' and have country tiled roofs. The grain markets are anything but sanitary and all godowns are heavily rat-infested. Recent extensions in Chaderghat area are quite hygienic and in fact compare favourably with any modern city. The City Improvement Board is very active in replacing slum areas with model dwellings.

Climate and rainfall—The climate is warm The average mean temperature is 91°F, December being the coldest month with an average mean temperature of 61°F, and May being the warmest month with an average mean temperature of 105°F The average rainfall is 31 inches

Exports and imports—Besides being the capital of the dominion it is an important trading centre The staple food of the people is rice, chiefly imported from the surrounding Telangana districts, as well as from Bezwada and the adjoining districts in the Madras Presidency Some years back rice was imported from Rangoon but for commercial reasons this has stopped The city has trade communications with Bombay, Poona, Sholapur, Madras, Cawnpore, Nagpur, Raipur and many places in the Punjab Most of the trade used to pass through Bombay, Poona and Sholapur (all plague-infected places), for these were the first places to be connected by railways with Hyderabad Later connection was established with Madras through M S M Railway at Bezwada, but the trade passing through this line was small as compared with that from the Bombay side via Wadi It is only within the last few years that direct connection has been established between this city and other places in southern India by the new railway extension from Secunderabad to Kurnool Similarly with Central and Upper India by the Kazipet-Ballarsha extension

History of plague in Hyderabad City—In spite of the free communication existing with plague infected places such as Bombay, Poona and Sholapur, plague did not make its appearance till the year 1911 When once it broke out it took a heavy toll, the first epidemic alone (31st August, 1911 to 28th April, 1912) being responsible for 18,478 attacks and 16,901 deaths The following table gives a brief history of plague in this city —

TABLE I

Period of epidemic	Attacks	Deaths
1911-1912	18,478	16,901
1915-1916	16,983	14,980
1918-1919	2,414	1,834
1919-1920	6,330	5,148
1920-1921	943	694
1923-1924	248	181
1924-1925	7,600	6,301
1925-1926	3,437	2,554
1926-1927	260	194
1927-1928	6,254	5,015
1928-1929	1,335	905
1929-1930	599	410
1930-1931	1,780	1,132
TOTAL	66,661	56,249

It will be observed from the above table that after the first epidemic (1911-1912) the city was free for the next three years. The second epidemic broke out in the year 1915 and was almost as severe as the first. After an interval of two years plague made its appearance again in the year 1918, but this epidemic was comparatively a mild one. The epidemic continued regularly for two years. There was no epidemic during the year 1922. From the year 1923 onwards the epidemic has appeared regularly each year. From Table II given below it is seen that the epidemic becomes established in September, reaches its height during the months of November, December and January, and subsides completely by the middle of April.

In the year 1929 (September) on the recommendation of the senior author (J N W) of this paper a Special Plague Department was started by H E H the Nizam's Government with the idea of carrying out an extensive rat campaign for the first time in the city of Hyderabad, and also to take over and improve the existing anti-plague measures.

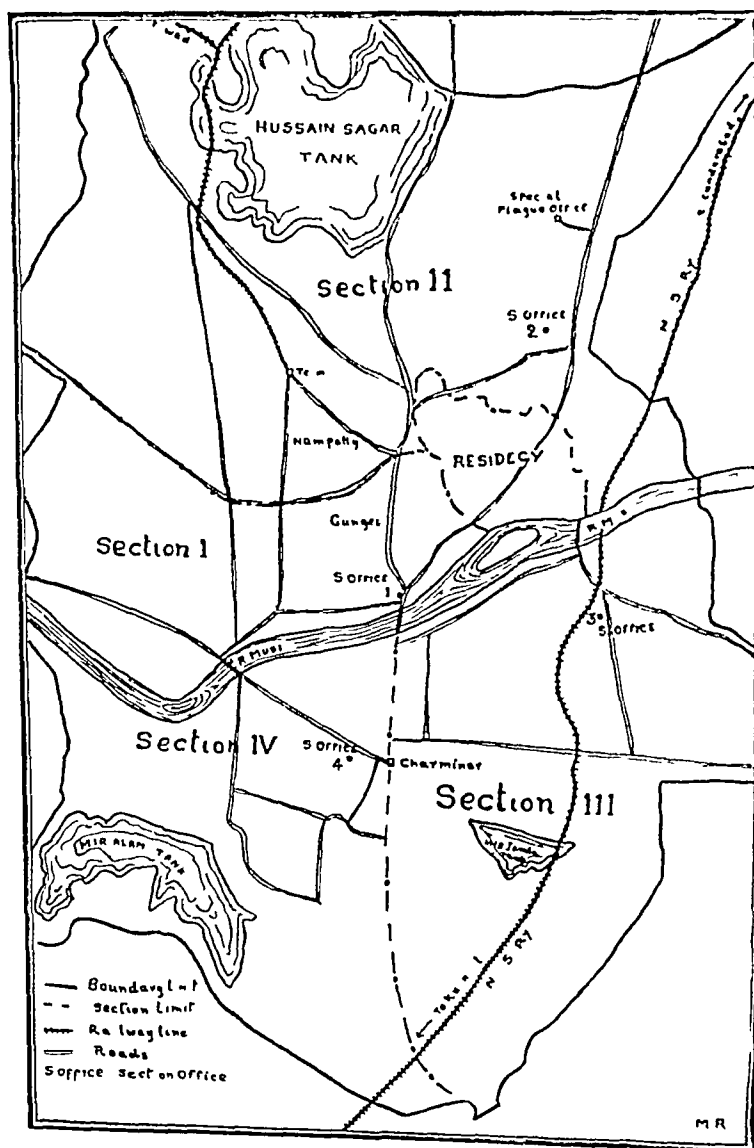
The anti-plague work included a rat-flea survey of the city. The work was started from September 1929. For this work the city of Hyderabad was divided into four sections, two on the northern side and two on the southern side of the river Musi (*vide* Map 1). Each section was placed under a Section Officer (of Sub-assistant Surgeons grade) and he was assisted in his work by four sanitary sub-inspectors, and a batch of Kamaties and Kamatans (men and women coolies).

The rat-density in the beginning of the campaign (*vide* Table III) in the month of September 1929 was 68.1 in Section I, 26.8 in Section II, 25.7 in Section III, and 35.1 in Section IV, while in the grain godown areas (Gunjes) it was 127.6. From September 1929 to March 1931 the total number of traps laid and rats caught was 1,754,459 and 317,744 respectively. The density of rats varied in all sections during the different parts of the year. It was high during the monsoon period (June to the middle of October). From October onwards the reduction in density was gradual till it reached its lowest limit during the month of March. Besides seasonal effect on the breeding of the rats, another contributing factor towards this variation of number of rats caught per hundred traps set is the baiting by barium carbonate pills. Baiting is usually stopped during the plague season (July onwards) as the presence of a large number of dead rats during this time of the year creates a panic among the public. This stoppage of baiting generally increases the number of live rats caught in traps. Similarly during the off-seasons a vigorous campaign of baiting is carried on throughout the city and this decreases the number of live rats trapped. By the end of March 1931 (after two years of anti-rat campaign) there is a tremendous decrease in the density of rat population, the figures for this month for the number of rats caught per 100 traps set being 6.1 in Section I, 15.7 in Section II, 2.5 in Section III, and 7.3 in Section IV, whilst in the Gunjes it was 16.7. These figures compare very favourably with the figures

at the beginning of the anti-rat campaign Baiting was found to have a marked effect on the density of rat population when carried on for two or three months at a stretch. This reduction in the density of rats was more marked in the year 1931

MAP 1

Map of Hyderabad City showing different sections



than in the year 1930. The density was lowest particularly in those sections where plague was more virulent in the year 1930-1931 (Sections III and IV). Up to 6th October, 1931, only those rats were classified that were examined for fleas. But from 6th October onwards all the rats caught in the city were classified, the

TABLE III

Month and Year	SECTION I *			SECTION II			SECTION III			SECTION IV			GRAIN MARKETS (Gunjes)		
	Number of traps laid	Number of rats caught	Rat density, i.e., number of rats per 100 traps	Number of traps laid	Number of rats caught	Rat density	Number of traps laid	Number of rats caught	Rat density	Number of traps laid	Number of rats caught	Rat density	Number of traps laid	Number of rats caught	Rat density
September 1929	11 385	7,764	68.1	5,602	1 448	25.8	6,614	1,702	25.7	2,824	992	35.1	3 054	3 897	127.6
October 1929	13 861	4 299	31.0	9 483	2 183	23.0	10,791	3 480	32.1	7 688	2 601	33.8	3,535	2,336	66.0
November 1929	21,856	3 744	17.1	14 389	1 864	12.9	17,627	3,397	19.2	13,750	2 164	15.7	4,620	1,300	28.1
December 1929	17,402	2 757	15.8	13 782	1 689	12.2	14,324	2,631	18.2	11,000	1 938	17.5	6,060	883	14.5
January 1930	27 594	3 127	11.3	21 402	2 399	11.2	22 340	3 931	17.6	17 500	3,706	21.1	6 060	861	14.2
February 1930	17 116	1 726	10.0	14 502	1 386	9.5	14 741	2 799	18.9	11 500	1 865	16.2	5 451	707	12.9
March 1930	18 511	1 721	9.3	17 288	2,021	11.6	17 560	3 279	18.6	13 000	2 253	17.3	4 900	564	11.5
April 1930	23 800	2 786	11.7	21 224	2 580	12.1	23 590	4 149	17.6	18 950	3 968	20.9	5 424	1 121	20.7
May 1930	16 800	2 566	15.2	14 780	2 212	14.9	15 502	2 648	17.0	14 800	2 052	13.8	4 725	1 208	25.5

June	1930	22,400	5,061	22 5	22,480	3,650	16 2	22,096	3,895	17 6	20,100	3,462	17 2	4,900	1,874	38 1
July	1930	19,600	7,281	37 1	19,510	4,302	22 0	21,325	5,225	24 4	20,500	5,538	27 0	5,425	2,654	48 9
August	1930	23,750	8,237	34 6	19,280	4,794	24 8	33,490	9,642	28 8	20,975	5,714	27 2	6,000	3,037	50 6
September	1930	28,900	9,068	31 3	22,075	5,054	22 8	40,770	11,656	28 5	32,400	7,897	24 3	6,000	2,036	33 9
October	1930	31,400	10,676	34 0	21,000	5 518	26 2	34,310	8,060	22 3	29,700	7,558	25 4	8,250	3,755	45 5
November	1930	39,700	10,741	27 0	25,500	7 354	28 8	42,120	6,014	14 2	28,700	6,054	21 0	12 000	3,842	32 0
December	1930	44,800	8,994	20 0	27 800	5,946	21 3	45,360	2,784	6 1	31 150	3,730	11 9	12,000	3,341	27 8
January	1931	55,450	7,953	14 3	27,880	5,085	18 2	37,560	1,396	3 7	27,600	1,823	6 6	15,000	1 785	11 8
February	1931	47,150	3,185	6 7	23,000	3,677	15 9	31,680	626	1 9	21,950	1,187	5 4	15,420	642	4 1
March	1931	54,410	3,363	6 1	32,000	5,052	15 7	34 730	885	2 5	15,300	1,180	7 3	11,100	1,852	16 7
TOTAL		535,885	105,049	19 6	372,977	68,214	18 2	486,230	78,799	16 2	359 387	65,682	18 2	139 928	37,698	26 9

* The figures in this section include also the figures from the grain markets given separately in the last three columns of this table
N B—The reduction in the rat density is not entirely due to trapping—large number of rats have been destroyed by barium carbonate baits in addition to regular trapping

results are given in Table IV. Out of 115,475 rodents trapped, 85,906 were *Rattus rattus*, 28,768 were mice, 682 were musk rats, 65 were *Gunomys varius* and 54 were bandicoots. The proportion of males to females in *Rattus rattus* and mice was roughly 1 : 3, while in musk rats and bandicoots the proportion was roughly 1 : 4. In *Gunomys varius* there was a slight excess of males over females.

The highest rat-density is in Section I which contains most of the grain markets and godowns. In fact the rat-density in the Gunjes has always been greater than in any other place in the city.

FLEAS

Details are given in Tables V and VI. Each section officer daily sent rats to the laboratory for flea survey work. Only those rats were selected that were caught singly in traps. Care was taken to see that all traps used were clean and only baits used that could not give shelter to fleas. Traps were collected early in the morning and were covered immediately with white flea-proof drill bags. These bags reached the laboratory before 10 A.M. Here they were transferred into an airtight wooden box and chloroformed to kill the rats and fleas. The bags were opened on a table covered with white mackintosh and all the fleas from the bags were picked up and laid aside. The rats were next combed, brushed and lastly thoroughly beaten on the table so that no fleas may remain on their bodies. All the rats examined belonged to *Rattus rattus* variety. Table V gives the figures for rats examined and fleas found for each month separately. In the succeeding columns of the same table the general flea-index, *cheopsis*-index for *Rattus rattus* and *astria*-index for *Rattus rattus*, are given. In the last three columns (9, 10, 11) the mean maximum temperature and relative humidity are given. In the next table (Table VII) the relationship of flea-index to the severity of plague epidemic is shown for the corresponding months.

Flea-index was highest during the months of September and October (6.4 and 6.2 respectively) when the mean maximum temperature fell to 88°F and the humidity was at its maximum for the year (80). A low temperature and a high percentage of humidity (the two favourable conditions for breeding of fleas) were present during these two months. This rise of flea-index has always coincided with the plague epidemic. Similarly during the months of April, May and June the temperature was high (mean maximum temperature for these months being 100°F, 104°F and 99°F respectively) and the atmospheric humidity at a very low percentage (mean humidity at 8 A.M. for these months being 53, 56, and 57 respectively). These conditions being unfavourable for the breeding of fleas account for the low flea-index during these months. This low flea-index coincides with the cessation of the plague epidemic and the subsequent off-season.

TABLE V

Month and Year	Number of <i>R. rattus</i> examined	Number of fleas obtained	General flea index	<i>Xenopsylla cheopis</i>	<i>Cheopsis</i> index for <i>R. rattus</i>	<i>Xenopsylla astia</i>	<i>Astia</i> index for <i>R. rattus</i>	Mean maximum temperature	Mean minimum temperature	Humidity
1	2	3	4	5	6	7	8	9	10	11
October 1929	62	172	2.8	138	2.2	34	0.5	89.4°F	69.0°F	71
November 1929	236	547	2.3	524	2.2	23	0.1	87.9°F	62.9°F	64
December 1929	167	521	3.0	504	3.0	17	0.1	86.3°F	62.9°F	71
January 1930	216	654	3.0	615	2.8	39	0.2	88.2°F	60.8°F	67
February 1930	177	190	2.8	471	2.7	19	0.1	89.0°F	62.0°F	50
March 1930	294	508	1.7	478	1.6	30	0.1	98.6°F	69.9°F	50
April 1930	529	628	1.2	575	1.1	53	0.1	100.3°F	75.5°F	53
May 1930	393	315	0.8	289	0.7	26	0.1	104.3°F	81.3°F	50
June 1930	408	261	0.6	235	0.5	26	0.1	99.2°F	76.5°F	67
July 1930	196	575	2.9	538	2.7	37	0.06	89.9°F	73.0°F	74
August 1930	203	800	3.9	745	3.6	55	0.3	88.6°F	73.2°F	75
September 1930	136	867	6.4	834	6.1	33	0.2	88.3°F	72.4°F	80
October 1930	107	666	6.2	628	5.9	38	0.3	87.0°F	70.7°F	80
November 1930	110	578	5.2	566	5.1	12	0.1	84.2°F	62.6°F	71
December 1930	108	587	5.4	555	5.1	32	0.3	85.7°F	60.5°F	77
January 1931	81	266	3.3	223	2.7	43	0.5	85.1°F	67.3°F	69
February 1931	35	169	4.8	156	4.5	13	0.3	93.2°F	64.9°F	69
March 1931	113	199	1.7	162	1.4	37	0.3			.
Total	3,571	8,803	2.4	8,236	2.3	567	0.1			

Total fleas examined were 8,803, out of these 8,236 were *Xenopsylla cheopis* and the rest (567) were *Xenopsylla astia*. Percentage of *Xenopsylla cheopis* was 93.6 and that of *Xenopsylla astia* was 6.4. Table VI gives the sexes of fleas examined separately for each month. In case of *cheopis* females predominated over males throughout the year except during the months of February and March.

TABLE VI

Month and Year	Rats examined	Fleas found	<i>X. cheopis</i>		<i>X. astia</i>	
			Male	Female	Male	Female
October 1929	62	172	56	82	10	24
November 1929	236	347	220	304	8	15
December 1929	167	521	220	275	6	11
January 1930	216	654	294	321	16	23
February 1930	177	490	241	230	10	9
March 1930	294	508	209	269	11	19
April 1930	529	628	237	338	15	38
May 1930	393	315	134	155	9	17
June* 1930	408	261	93	142	11	15
July 1930	196	575	247	291	19	18
August 1930	203	800	329	416	15	40
September 1930	136	867	417	417	9	24
October 1930	107	666	272	356	15	23
November 1930	110	578	264	302	3	9
December 1930	108	587	273	282	19	13
January 1931	81	266	116	107	17	26
February 1931	35	169	85	71	6	7
March 1931	113	199	79	83	16	21
TOTAL	3,571	8,803	3,795	4,441	215	352

* One *Ctenocephalus felis* was found during this month on one of the rats.

TABLE VII

Month and Year		General flea index for <i>Rattus rattus</i>	Number of plague attacks
October	1929	2 8	45
November	1929	2 3	50
December	1929	3 1	62
January	1930	3 0	125
February	1930	2 8	187
March	1930	1 7	64
April	1930	1 2	21
May	1930	0 8	
June	1930	0 6	
July	1930	2 9	.
August	1930	3 9	7
September	1930	6 4	57
October	1930	6 2	126
November	1930	5 2	358
December	1930	5 4	535
January	1931	3 3	434
February	1931	4 8	230
March	1931 .	1 7	29

The close relationship of the high flea-index to the plague epidemic is obvious

when there was slight increase of males over females In case of *Xenopsylla astia* also the above fact holds good generally but in the months of February 1929 and December 1930 there was slight excess of males over females

TABLE VIII

Locality	Total <i>Rattus</i> <i>rattus</i> examined	FLAS FOUND ON <i>Rattus rattus</i>					
		<i>X cheopis</i>		<i>Cheopis</i> index	<i>A. astia</i>		<i>Astia</i> index
		Male	Female		Male	Female	
Narayenguda	143	262	252	3.6	38	60	0.70
Nampally	19	57	44	5.3	0	1	0.05
Troop Bazar	24	61	77	5.7	5	18	0.90
Goshamahall	20	60	37	4.8	8	11	0.90
Dhoolpet	32	102	95	6.1	0	1	0.03
Sitarambag	22	50	55	4.8	0	0	
Begum Bazar	49	138	129	5.4	1	3	0.03
Chowraha Jinsi	33	66	111	5.4	8	7	0.4
Osmanshal	28	62	52	4.1	0	0	
New Bridge	21	60	55	5.5	0	2	0.07
Pathargutti	23	50	56	4.6	1	2	0.1
Charminar	32	83	113	6.1	4	1	0.1
Ghansi Bazar	26	67	75	5.5	2	2	0.1
Chowk	23	42	68	4.8	2	1	0.1
Fathe Darwaza	16	60	62	7.6	3	4	0.4
Yakootpura	22	68	64	6.0	0	0	
Dabirpura	29	100	121	7.6	0	3	0.1
Malakpet	18	91	76	9.3	1	2	0.1
Kachiguda	21	94	101	9.3	0	0	
Lingumpally	19	58	66	6.5	2	11	0.7
Residency Bazars	253	406	440	3.3	3	2	0.2
TOTAL	853	2,037	2,149	4.9	78	140	0.25

TABLE IX

Fleas examined from different localities for specific flea-index.

Month and Year	Number of <i>Rattus rattus</i> examined	FLEAS FOUND ON <i>Rattus rattus</i>				Number of mice examined	FLEAS FOUND ON MICE			
		<i>X cheopis</i>	<i>Cheopis</i> index for <i>Rattus rattus</i>	<i>X astia</i>	<i>Astia</i> index for <i>Rattus rattus</i>		<i>X cheopis</i>	<i>Cheopis</i> index for mice	<i>X astia</i>	<i>Astia</i> index for mice
July 1930	69	318	4.6	8	0.1	10	37	3.7	1	0.1
August 1930	100	681	6.8	52	0.5					
September 1930	117	831	7.1	33	0.3					
October 1930	77	508	6.6	20	0.3					
November 1930	93	420	4.5	15	0.2					
December 1930	54	278	5.1	10	0.2					
January 1931	38	108	2.8	30	0.8	8				
February 1931	20	105	5.2	13	0.6	2	4	2.0		
March 1931	52	91	1.7	32	0.6	12	7	0.6		
TOTAL	620	3,340	5.4	213	0.3	32	48	1.5	1	0.03

Flea-index in the wheat markets (Gunjes)

Gunjes	Number of <i>Rattus rattus</i> examined	<i>X cheopis</i>	<i>Cheopis</i> index	<i>X astia</i>	<i>Astia</i> index
Osmangunj	54	196	3.6	11	0.7
* Mukhtargunj	20	85	4.2	22	1.1
Mahaboobgunj	18	84	4.8	17	0.9
Kishengunj	12	23	1.9	9	0.7
Maharajgunj	42	121	2.9	39	0.9
TOTAL	146	509	3.4	128	0.9

* On 12.3.30 one *Ceratophyllus fasciatus* was found in this Gunj

During the course of our work we found few *X astia* on rats brought from different localities. To locate these particular areas the following procedure was adopted. An Inspector with eight Kamaties was detailed with 100 traps to collect rats from different portions of the city in a regular order. The whole city was divided into fourteen sections corresponding with the municipal wards, each locality was trapped for two days in rotation. Thus each locality was trapped roughly once a month. All these traps were brought to the laboratory and only those traps containing a single rat were selected. These traps, like the previous ones, were covered immediately they were taken out of the houses, with white flea-proof drill bags. In the laboratory the rats were examined as usual, fleas collected and marked separately according to their species and sexes. The rats also were classified. Tables VIII and IX give the number of rats and fleas thus examined from each locality. From Table VIII it can be seen that the localities from which the *X astias* were frequently found were Narayenguda, Troop Bazar, Goshamahall and Lingumpally. The other places where also the *X astia* was frequently found was the Gunj (gram markets) area. The result of flea examination of the Gunjes is given above (see Table VIII).

During the whole period of survey only two *Ctenocephalus felis* and one *Ceratophyllus fasciatus* were found on rats. The accompanying map Map 2 of Hyderabad City shows the distribution of *X cheopis* and *X astia* in different localities.

MAP 2

Map of Hyderabad City showing the distribution of *X. astia*
and *X. cheopis*



SUMMARY

- (1) Period of survey was from September 1929 to March 1931
- (2) Total number of traps laid were 1,754,479 Out of a total number of 317,744 rodents caught, 115,475 (rats caught after 6th October, 1930) were classified Out of these 85,906 were *R. rattus*, 28,768 were mice, 682 were musk rats, 65 were *Gunomys varius* and 54 were bandicoots

(3) The rat-density was highest in the gram markets

(4) Total number of fleas collected was 13,746 Of these 12,949 were *X cheopis*, 794 were *X astia*, two were *Ctenocephalus felis* and one *Ceratophyllus fasciatus*

(5) Plague appeared in Hyderabad in the year 1911 From the year 1924 it has recurred regularly every year

(6) Plague season in Hyderabad is from September to April and off-season from May to August

ANALYSES AND CALORIFIC VALUES OF SOME INDIAN FOOD-STUFFS

BY

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[Received for publication, June 22, 1931]

THOUGH many analyses and calorific values are obtainable from standard textbooks, as far as European countries and America are concerned the literature is still meagre as far as Indian food-stuffs are mentioned. In fact, apart from Captain MacCay's figures published in Bengal Jail Dietaries, 1910, we are aware of little of real value. The publication, therefore, of the following tables of analyses may prove of interest to all those whose duty or inclination concerns Hospital administration or the study of dietetics in India. The chemical analyses were carried out in the School of Tropical Medicine, the calorific values in the laboratory of the Chemical Examiner to the Government of Bengal. The object in the first instance was to determine calorific values, but the chemical analyses were necessary to supplement these.

Table I deals with milk analysis, the tables themselves are self-explanatory.

TABLE I

Milk analysis

Sample No		Fat per cent	Total solids	Solid not fat	Ash per cent	CaO per cent	Alkalinity	Calorific value (in large calories) on dried samples 1 gramme
COWS	1	5.6	15.26	9.66	0.72	0.21	24.4	6.22
	2	4.9	14.12	9.22	0.66	0.18	22.6	6.15
	3	5.5	14.78	9.28	0.67	0.18	23.6	6.09
	4	5.3	14.93	9.62	0.75	0.20	19.2	6.27
	5	6.3	16.05	9.75	0.80	0.21	19.2	6.31
	6	1.6	11.84	10.24	0.83	0.20	18.9	5.16
	7	5.2	14.94	9.74	0.75	0.21	19.5	6.16
BUFFALOES	8	12.3	21.42	9.12	0.73	0.21	12.8	7.22
	9	7.5	16.43	8.93	0.69	0.21	21.3	6.85
	10	8.0	16.91	8.91	0.74	0.21	25.3	6.98

TABLE II

Calorific value per gramme of original actual milk in big calories

Sample No *	Cows							BUFFALOES		
	1	2	3	4	5	6	7	8	9	10
Actuals	0.95	0.87	0.90	0.94	1.01	0.61	0.92	1.55	1.12	1.18
Calculated	0.96	0.87	0.94	0.93	1.03	0.61	0.93	1.57	1.11	1.15

* Samples Nos 1 to 3 are the figures for mixed samples

Samples Nos 4 to 5 are for individual animals

Sample No 6 is a sample of foremilk

Sample No 7 is from an individual animal

Samples Nos 8, 9 and 10 are from individual buffaloes

Alkalinity figure is the number of c.c. of N/10 acid required to neutralize the ash of 100 grammes of the sample

Calorific values are given in big calories on the formula

$$\frac{S}{100} \times 4.5 + \frac{F}{100} \times 9.4 = \text{Cal value of 1 gramme of original milk}$$

The next series of tables shows the analyses of samples of cow ghee three samples of which were done in duplicate, and also an analysis of the insoluble fatty acids carried out on the same samples. The last table in this series shows the calorific values obtained from these samples (Tables III, IV and V)

TABLE III

Analysis of samples of cow ghee

	(1)	(1)	(2)	(2)	(3)	(3)
Iodine value	34.2	34.3	30.2	30.8	27.9	28.1
Saponification value	226.5	227	227.7	228.3	226.5	227
Refractometer reading at 40°C		13.0		41.9		41.9
Sp. Gr. 99°/15.5°		0.914		0.914		0.911
Hehner value	90.1		90.9		89.2	
Reichert Wollny value	22.9		26.67		23.5	

TABLE IV

Analysis of insoluble fatty acids (cow ghee)

	(1)	(2)	(3)
Iodine value	37.5	37.2	30
Saponification value	222.1	221.4	218.1
Melting point	40°C	41°C	43°C
Titre value	38.8	39.5	40.1

TABLE V

Calorific values for cow ghee, in large calories per gramme

Samples	(1)	(1)	(2)	(2)	(3)	(3)
	9.41	9.41	9.39	9.41	9.41	9.40

The next series of tables shows the results obtained from analysis of samples of Burma rice, country rice and Balam rice. The average chemical compositions of the same as determined by Captain MacCay, I.M.S., are also shown and in addition the calorific values of the samples as received from the market and on the dried samples. The calorific values shown in Captain MacCay's tables are those determined by Benedict.

TABLE VI
Showing the average chemical composition
(Determined by Mr Bannerjee)
Rice

	Fat	Moisture	Ash	Protein	Carbohydrate by difference	Calorific value *	
						(1) and (2)	
Burma Rice	1 08	10 1	1 05	6 10	81 67	3 75	4 171
Country Rice	1 8	9 9	1 03	5 47	81 80	3 74	4 150
Balam Rice	1 2	10 4	1 04	7 12	80 24	3 79	4 179

(Taken from Capt MacCay's figures)

Burma Rice	0 96	11 13	1 34	6 95	77 25	3 820
Country Rice	0 86	11 05	1 32	6 86	78 85	3 824

* Calorific values (1) and (2) in large calories per gramme
(1) = Heat of combustion on the sample as received
(2) = Heat of combustion on the dried sample

Table showing the actual calorific values as determined by the Mahler Bomb calorimeter

TABLE VII

	Calorific value of samples as received in large calories per gramme		Calorific value of dried sample in large calories per gramme
Burma Rice determined on three samples	{ 3 714 3 742 3 734 3 790 3 762 3 756 }	Average = 3 75	{ 4 171
Country Rice determined on three samples	{ 3 725 3 713 3 787 3 780 3 730 3 724 }	Average = 3 74	4 150
Balam Rice determined on three samples	{ 3 780 3 745 3 788 3 784 3 818 3 871 }	Average = 3 797	{ 4 173 4 185

The next series of tables deal with atta (white and brown), the figures obtained by Captain MacCay are also shown. Calorific values as obtained from the actual samples (1) and also after drying of the samples (2) are included.

TABLE VIII

Atta

	Fat	Moisture	Ash	Protein	Carbohydrate by difference	Calorific values* (in large calories per gramme)	
						(1)	(2)
Atta (white) ..	1 06	11 1	0 69	7 8	79 35	3 83	4 16
Captain MacCay's figures	2 18	11 83	2 43	12 24	70 92	4 017	
Atta (brown)	2 4	11 09	1 98	9 8	74 73	3 98	4 36

* (1) Determined on substance as received (large calories per gramme)

(2) " " " " dried " " " "

TABLE IX

Atta

	Calorific values of samples as received (in large calories per gramme)	Calorific values of dried samples (in large calories per gramme)
Atta (white) determined on three samples	<div><div>3 818 3 835 3 872 3 821 3 843 3 841</div><div>Average = 3 83</div></div>	<div><div>4 144 4 177</div><div>Average = 4 366</div></div>
Atta (brown) determined on seven samples	<div><div>3 837 3 786 3 905 3 921 3 980 3 956 3 839 3 836 3 944 3 928 3 982 4 009 3 921 3 938</div><div>Average = 3 98</div></div>	<div><div>4 319 4 367 4 314 4 415 4 450 4 420 4 340 4 307</div><div>Average = 4 366</div></div>

The next series of tables are concerned with various varieties of dal both as regards analytical figures and actual calorific values Captain MacCay's figures are also given

TABLE X

Dal

	Fat	Moisture	Ash	Protein	Carbohydrate	Calorific value of samples as received (in big calories)	Calorific value of dried samples (in big calories)
Gram Dal	6 35		2 15	20 57		4 16	4 64
Mattar Dal	1 96	8 17	2 68	22 28	64 91	3 81	4 14
Mussur Dal	1 08	8 51	1 95	24 69	63 77	3 82	4 15
Sonamug Dal	1 75	8 7	3 72	25 95	59 88	3 92	4 4
Arhar Dal	1 52		3 94	22 78		3 86	4 2
Kala Dal	1 37	9 34	3 44	21 02	64 83	3 84	4 37

Similar samples with Capt MacCay's analysis

Gram Dal	4 31	10 07	3 72	19 94	51 13	4 285
Mattar Dal	1 96	10 96	3 60	22 01	53 97	4 075
Mussur Dal	3 00	10 23	3 33	25 47	55 03	4 059
Sonamug Dal	2 69	10 87	3 57	23 62	53 45	4 051
Arhar Dal	3 33	10 08	5 50	21 67	54 27	4 083
Kala Dal	1 10	10 87	3 61	22 58	58 02	4 038

Table showing the actual calorific value as determined on the Mahler Bomb calorimeter

TABLE XI

Dal

	Calorific value of samples as received (large calories per gramme)	Calorific value of dried samples (large calories per gramme)
Gram Dal determined on three samples	$\left\{ \begin{array}{l} 4\ 206 \\ 4\ 212 \\ 4\ 177 \\ 4\ 171 \\ 4\ 099 \\ 4\ 074 \end{array} \right\}$ Average = 4 110	$\left\{ \begin{array}{l} 4\ 617 \\ 4\ 633 \end{array} \right\}$
Mattar Dal determined on three samples	$\left\{ \begin{array}{l} 3\ 814 \\ 3\ 811 \\ 3\ 809 \\ 3\ 810 \\ 3\ 807 \\ 3\ 822 \end{array} \right\}$ Average = 3 81	4 142
Mussur Dal determined on three samples	$\left\{ \begin{array}{l} 3\ 813 \\ 3\ 764 \\ 3\ 844 \\ 3\ 842 \\ 3\ 848 \\ 3\ 834 \end{array} \right\}$ Average = 3 82	4 150
Sonamug Dal determined on three samples	$\left\{ \begin{array}{l} 3\ 955 \\ 3\ 959 \\ 3\ 926 \\ 3\ 928 \\ 3\ 876 \\ 3\ 888 \end{array} \right\}$ Average = 3 92	$\left\{ \begin{array}{l} 4\ 440 \\ 4\ 451 \end{array} \right\}$
Arhar Dal	$\left\{ \begin{array}{l} 3\ 821 \\ 3\ 844 \\ 3\ 879 \\ 3\ 895 \\ 3\ 878 \\ 3\ 858 \end{array} \right\}$ Average = 3 86	4 218
Kalai Dal	$\left\{ \begin{array}{l} 3\ 817 \\ 3\ 836 \\ 3\ 840 \\ 3\ 807 \\ 3\ 907 \\ 3\ 859 \end{array} \right\}$ Average = 3 84	$\left\{ \begin{array}{l} 4\ 374 \\ 4\ 366 \end{array} \right\}$

The next series of tables deal with mustard oil and ground nut oil showing analysis of the oil obtained from various samples, the analysis of the insoluble fatty acids and the calorific values obtained

In these samples the only object was to obtain calorific values, and the detailed chemical analyses are supplementary to this. In the sample of mustard oil the iodine values found are somewhat on the high side.

TABLE XII

Chemical analysis of samples of mustard oil

	(1)		(2)		(3)	
Saponification value	171.1	171.2	171.8	172.6	171.8	172.3
Iodine value	107.2	107.6	105.4	105.7	106.2	106.6
Refractometer reading at 40°C	59.8		58.7		59.0	
Sp. Gr. 15.5°C / 15.5°C	0.920		0.921		0.921	
Hehner value	94.5		93.8		94.0	

TABLE XIII

Analysis of insoluble fatty acids (mustard oil samples)

	(1)		(2)		(3)	
Iodine value	109.5	110.0	105.9	105.9	109.2	110.0
Saponification value	177.9	178.5	179.1	179.2	178.0	178.2
Melting point	20.5		19.5		19.5	
Titre value	17.9		17.0		17.5	

TABLE XIV

Calorific values obtained on samples of the oil (in small calories per gramme)

	(1)		(2)		(3)	
	9,726.6	9,755.0	9,917.5	9,872.0	9,743.5	9,810.0

TABLE XV

Chemical analysis of samples of ground nut oil

	(1)		(2)		(3)	
Saponification value	191.7	191.9	187.0	187.1	187.0	187.2
Iodine value	86.0	86.2	89.3	89.5	89.1	89.4
Refractometer reading at 40°C	53.7		55.4		51.9	
Sp. Gr. 15.5°C/15.5°C	0.915		0.917		0.917	
Hehner value	95.7	95.9	96.0	96.2	96.3	96.3

TABLE XVI

Analysis of insoluble fatty acids (ground nut oil)

	(1)		(2)		(3)	
Iodine value	89.4	89.8	94.0	93.9	93.0	92.9
Saponification value	200.0	200.0	195.9	196.3	192.8	193.2
Melting point	33-37		34-37		35-37	
Titre value	27.9		30.0		29.0	

TABLE XVII

Calorific values obtained on samples of the oil (ground nut oil) in small calories per gramme

9,618.2	9,622.6	9,649.6	9,728.9	9,582.4	9,637.4
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The next series of tables deal with samples of niger seed oil, white and black sesame seed oil, the analysis of the samples the insoluble fatty acids and the calorific values obtained

TABLE XVIII
Chemical analysis of niger seed oil samples

	(1)		(2)		(3)	
Iodine value	139 1	139 5	138 9	139 4	140 0	140 4
Saponification value	190 5	191 1	192 1	192 5	192 8	193 1
Refractometer reading at 40°C	63 3		63 0		63 2	
Hehner value	96 3	96 3	96 3	96 4	96 0	96 0
Sp Gr 15.5°C/15.5°C	0 925		0 925		0 925	

TABLE XIX
Insoluble fatty acids (niger seed oil)

	(1)		(2)		(3)	
Iodine value	143 7	144 4	143 3	143 3	145 5	145 5
Saponification value	194 7	195 2	197 5	197 7	199 0	199 1
Melting point	27 0		27 0		27 0	
Titre value	23 5		23 3		23 6	

TABLE XX
Calorific values obtained on the samples in small calories per gramme (niger seed oil)

9,563 5	9,594 1	9,497 1	9,485 7	9,488 0	9,578 9
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TABLE XXI

Chemical analysis of samples of white sesame seed oil and black sesame seed oils

	White		Black			
			(1)		(2)	
Iodine value	108.3	108.8	109.7	109.5	108.6	109.4
Saponification value	192.1	192.7	190.0	190.3	193.8	191.0
Refractometer at 40°C	58.9		59.8		59.1	
Sp. Gr. 15.5°C /15.5°C	0.923		0.923		0.922	
Hehner value	95.7	95.8				

TABLE XXII

Insoluble fatty acids of white and black samples of sesame seed oil

	White		Black			
			(1)		(2)	
Iodine value	112.6	113.2	109.0	109.0	112.1	112.4
Saponification value	201.3	202.0	202.0	202.8	198.8	199.2
Melting point	28.5		28.29		28.0	
Titre value	23.1		24.0		24.0	

TABLE XXIII

Calorific values of the samples in small calories per gramme

White variety	9,625.2	9,655.8		
Black variety	9,467.2	9,485.9	9,669.7	9,660.0

The following tables are the result of an analysis of ordinary diets (European, Hindu and Mahommedan), as they were issued to patients without any extras in a large general hospital. The detailed analyses are given as well as the calorific values

determined on actual cooked samples. It is interesting to note that the European ordinary diet is lowest in calorific value, the Mahommedan being highest. The comparative totals of the individual basal items are also of interest.

TABLE XXIV

	European	Hindu	Mahommedan
Ash	15.57 grammes	19.16	19.23
CaO	0.899 „	1.26	1.25
Protein	81.36 „	80.9	87.4
Fat	57.64 „	30.3	38.1
Calorific value	2,114.6 „	2,594.5	2,637.0

The European diet has the highest fat content but is much lower in carbohydrate and in calcium. The extra calories in the case of the Mahommedan diet are produced mainly by the greater amount of protein and fat.

TABLE XXV

Analysis of hospital diets (Mahommedan) obtained from cooked samples

Article	No	Quantity	Ash content	CaO content	Protein content	Fat content	Moisture per cent
Rice country	3	48 oz	1.63	0.68	17.6	5.4	77.68
Dal	10	18 „	10.7	0.1	36.7	2.0	81.50
Fish (fried) or	2	2½ „	2.5	0.06	18.9	4.1	60.3
Beef	1	7 „	2.4	0.07	20.8	23.6	79.2
Vegetables	2	4 „	2.4	0.09	1.1	3.2	85.9
Brown bread	2	4 „	1.30	0.11	8.4	8.2	20.1
Sugar	2	¾ „					
Milk	7	4 „	0.80	0.21	3.8	5.5	85.5
TOTALS			19.23	1.25	87.4	38.1	

TABLE XXVI

Calorific values of above diet

Article	No	Quantity	Calorific value (in large calories)
Rice, country	3	48 oz	1,281.7
Dal	10	18 "	117.5
Fish (fried) or Beef	2 1	2½ " 7 "	171.3 270.1
Vegetables	2	4 "	73.3
Brown bread	2	4 "	451.7
Sugar	2	¾ "	84.2
Milk	7	4 "	101.9
TOTAL			2,637.0

TABLE XXVII

Analysis of hospital diets (Hindu) obtained from cooked samples

Article	No	Quantity	Ash content	CrO content	Protein content	Fat content	Moisture per cent
Rice, country	3	48 oz	1.63	0.68	17.6	5.4	77.7
Dal	10	18 "	10.7	0.10	36.7	2.0	81.5
Fish and Soup or Fish (fried)	2 2	5 " 2½ "	3.5 2.5	0.08 0.06	7.8 18.9	8.0 4.1	78.0 60.3
Vegetables	2	4 "	2.4	0.09	1.1	3.2	85.9
Brown bread	2	4 "	1.3	0.11	8.4	8.2	20.4
Sugar	2	¾ "					
Milk	7	4 "	0.83	0.21	3.8	5.5	85.5
TOTALS			19.16	1.26	80.9	30.3	

TABLE XXVIII
Calorific value of diet

Article	No	Quantity	Calorific value (in large calories)
Rice, country	3	48 oz	1,281 7
Dal	10	18 "	417 5
Fish and Soup or Fish (fried)	2	5 "	190 5
	2	2½ "	171 9
Vegetables	2	4 "	73 3
Brown bread	2	4 "	454 7
Sugar	2	¾ "	84 2
Milk	7	4 "	101 9
TOTAL			2,594 5

TABLE XXIX
Analysis of hospital diets (European) obtained from cooked samples

Article	No	Quantity	Ash content	CaO content	Protein content	Fat content	Moisture per cent
Rice	3	12 oz	0 40	0 160	4 40	1 3	77 69
Dal	3	3 "	1 8	0 020	11 0	0 04	81 5
Vegetables	3	8 "	4 7	0 170	2 2	6 3	86 0
Butter	2	¾ "	0 34	0 000	0 16	17 24	16 2
Potatoes	3	1½ "	0 26	0 017	0 9	0 08	80 0
Pudding	1	90 gs	0 9	0 050	7 6	0 88	43 0
Sugar	3	2 oz					
Milk	7	4 "	0 83	0 210	3 8	5 5	35 6
Bread	2	6 "	2 04	0 156	15 60	3 4	
Mutton and Fish	2	5 "	2 10	0 057	14 4	17 3	60 0
Fish or Fowl and Beef	3	4 "	3 96	0 09	30 1	6 6	81 1
Beef or Beef and Fish	2	4 "	1 47	0 047	14 1	12 1	79 2
	1	5 "	1 69	0 054	14 8	14 8	79 2
	1	5 "	1 69	0 054	14 8	14 8	60 0
	3	2 "	1 98	0 045	15 0	3 3	85 5
TOTALS			15 57	0 899	81 36	57 64	

TABLE XXX.

Calorific values of above diet.

Article	No	Quantity	Calorific value (in large calories)
Rice	3	12 oz	330 9
Dal	3	3 „	68 5
Vegetables	3	8 „	115 8
Butter	2	3 „	167 7
Potatoes	3	1½ „	36 5
Pudding	1	90 gs	192 1
Sugar	3	2 oz	224 7
Milk	7	4 „	101 9
Bread	2	6 „	481 1
Mutton and	2	5 „	138 5
Fish	3	4 „	280 8
Fowl and	2	4 „	141 2
Beef	1	5 „	197 2
Beef and	1	5 „	197 2
Fish	3	2 „	140 4
TOTAL			2 114 6

THE ERYTHROCYTE SEDIMENTATION RATE IN KALA-AZAR.

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[Received for publication, June 29, 1931]

THE estimations, the results of which are reported below, were carried out by the junior writer during a study of the sedimentation rates of the erythrocytes in various diseases, especially with reference to the variations in this rate at different external temperatures. Although these estimations had been carried out in cases of a number of different diseases, including kala-azar, the results were recorded as a whole and no attempt was made to differentiate them according to diseases, so that when—in another connection—the senior writer wanted figures for the rate of sedimentation in kala-azar, reference had to be made to the junior writer's original notes, which were fortunately available.

It was felt that it would be worth while making these results of the sedimentation estimations in kala-azar cases available for future reference. In most instances a number of estimations were done on the blood of the same patient at different periods, before, during, and after treatment, we therefore decided to correlate these estimations with the records of the progress of the patients and other observed clinical features. As far as kala-azar patients are concerned no new estimations were carried out, because we did not feel justified in extending this inquiry. We discovered, however, that there were no records of sedimentation estimations

of the blood of normal persons by the method which had been adopted, and we therefore had such estimations in 30 apparently healthy individuals carried out for us by Mr N K De, B sc, the chemist attached to the leprosy department

The technique—The technique used in the leprosy research department of the School of Tropical Medicine and Hygiene, Calcutta, is an adaptation of that used by other workers and is chosen because it makes it possible to test a large number of bloods at once with fair accuracy and with the expenditure of a minimum of time. 0.3 c.c. of a 5 per cent solution of sodium citrate in distilled water is drawn into an all-glass 2 c.c. syringe, 1.2 c.c. of blood is drawn from the patient's median basilic vein into the same syringe, a small quantity of air is taken into the syringe barrel, the blood and the citrate solution are then thoroughly mixed by reversing the syringe several times, and the mixture is evacuated into a clean test-tube. If several patients are to be treated, their bloods are taken in a similar manner and placed in labelled test-tubes in a rack. Sedimentation is carried out in 300 mm pipettes, graduated from above downwards, from zero to 100, with a space of 3 mm between each mark. The content of the pipettes when filled to zero is approximately 1 c.c. but a variation of 0.05 c.c. is allowed, as such a variation makes no appreciable difference in the results. The pipettes are placed upright in a rack with their points inserted into small holes bored in rubber corks.

One of these pipettes is taken from the rack and its upper end attached to a 10 c.c. syringe by means of a rubber tube. The point of the pipette is inserted into one of the test-tubes and, suction being applied by pulling on the piston of the syringe, the blood-citrate mixture is drawn up into the pipette to the zero mark. The pipette is then replaced in the rack, the point is again inserted in the rubber cork, which prevents the mixture escaping, and the rubber tube is disconnected from the pipette. In this way the other pipettes are filled up to the zero mark from the other test-tubes.

The top level of the erythrocytes is read off after $1\frac{1}{2}$ hours and again after $2\frac{1}{2}$ hours, the average of these readings is taken as the sedimentation index. Thus, if the top level of the blood cells falls to 10 (30 mm) after $1\frac{1}{2}$ hours and to 20 (60 mm) after $2\frac{1}{2}$ hours the sedimentation index will be the average of 10 and 20, i.e.; 15. In the present series of observations the pipettes were in all cases kept in the incubator at a constant temperature of approximately 37°C .

The controls—These were (a) persons undergoing anti-rabic treatment who had no extensive or septic wounds, and (b) healthy servants at the Gobra Leper Asylum. Of these controls, two gave readings three times that of the mean of the whole group. One of these men was subsequently found, on enquiry, to be suffering from acute gonorrhoea, and the other from repeated attacks of malaria. These two

subjects are not normal, we consider, therefore, that we are justified in excluding these readings in calculating the mean

The kala-azar patients—These were all patients in the Carmichael Hospital for Tropical Diseases under the charge of the senior writer. In every instance the diagnosis had been made by demonstrating the parasite of the disease, either in the blood or in the spleen-puncture material, by direct or by cultural methods

THE RESULTS

(Recorded as sedimentation index units, see above)

Controls—The two estimations that were considered to be definitely abnormal were 50.0 and 48.25, of the remaining 28 the mean was 13.455, the standard deviation 7.101

Kala-azar—Of the 16 single estimations on 16 untreated kala-azar patients the mean was 67.675, and the standard deviation 15.665. The highest figure was 82 and the lowest 13. This last figure is exceptionally low, as the next highest figure is 54, if it is excluded the mean will be 71.320, and the standard deviation 7.509

It will be seen that the mean in the kala-azar cases is very far removed from the mean of the supposed-normal persons. In only one instance did a kala-azar patient give a reading lower than the highest of the supposed normals, this was a very early case of the disease in which all serum tests were negative, but in which the parasite had been recovered from the blood by culture

These observations might suggest that the estimation of the sedimentation rate of the red blood cells could be used as a diagnostic method in kala-azar. It is probably true that in no disease is the sedimentation rate so markedly accelerated as it is in kala-azar, but there are a number of conditions, some of which simulate kala-azar clinically, in which the sedimentation rate is also considerably accelerated

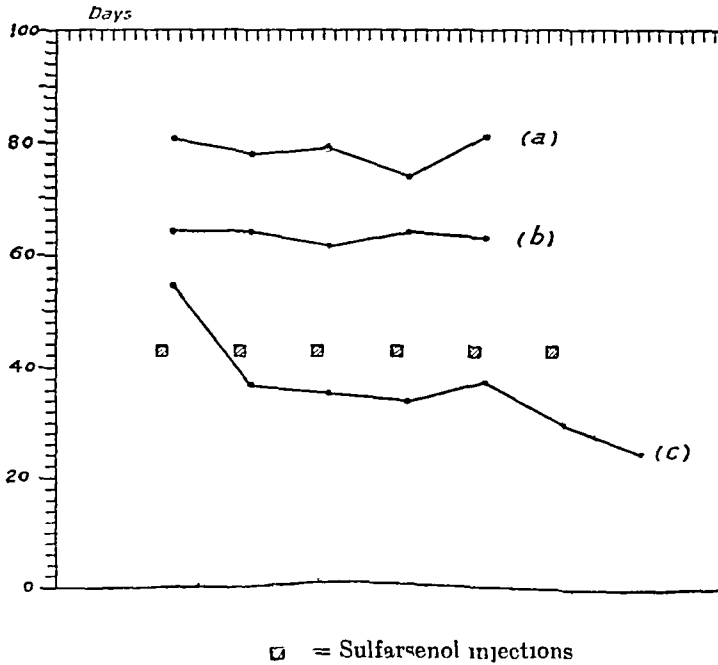
VARIATIONS IN THE SEDIMENTATION RATE IN THE SAME PATIENT

In three patients a number of estimations were done prior to the administration of antimony. The results are shown graphically on Chart 1. It will be seen that in the first two the sedimentation index is remarkably constant, the means being 78.60 and 63.34 and the standard deviations 2.60 and 1.04, respectively. The third was a congenitally-syphilitic patient. His Wassermann reaction was positive, so he was given a course of Sulfarsenol, after this most of his clinical symptoms of kala-azar disappeared, but as parasites were still found in his spleen puncture he was later given a course of injections and discharged. In this case it is

obvious that syphilis played a great part in the acceleration of the sedimentation rate

CHART 1

No antimony treatment



THE PROGNOSTIC SIGNIFICANCE OF THE SEDIMENTATION-RATE CURVE

The patients have been divided into two classes, those who progressed well clinically and were eventually cured, and those who did not progress well clinically and to whom further injections were given, or who relapsed

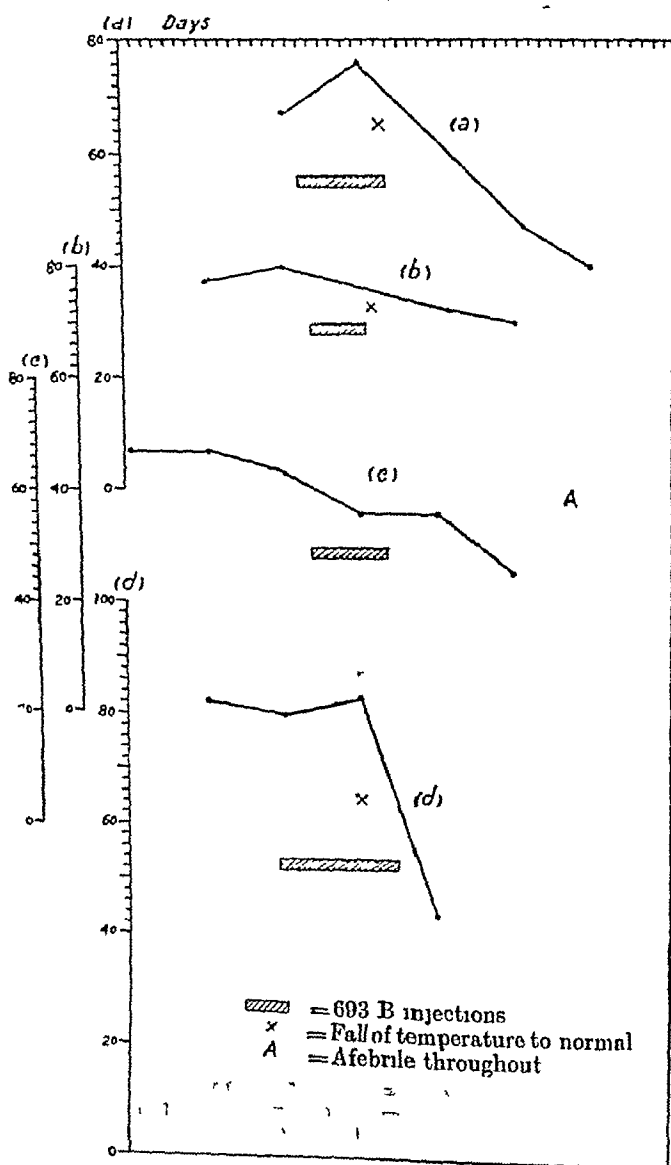
Single estimations—The mean of the single sedimentation-rate estimations in the former group is 66.91, or, if the case in which the estimation is 13 be excluded, 72.90, of the latter group the mean is 72.05. It is, therefore, clear that single estimations do not demonstrate any significant difference between the cases in the two groups

The sedimentation rate before and after treatment—In no instance did the sedimentation rate return to normal during the period of observation. Even in the cured-case group, excluding the one case in which the sedimentation index was only 13 before treatment, the mean of the final estimations was 50.20 units

In this series the estimations were not done at any constant interval after the completion of the course of antimony injections so that no very definite conclusions can be arrived at, but by comparing the first and last estimations in cases in which treatment was given it can be shown that there is a mean fall of 19.98 units for the patients who were cured by the treatment, and only 9.90 in those who did not

progress well clinically, or who relapsed. In one of the relapsing cases a drop of 32 units in the sedimentation rate was shown and in another in which rapid cure was effected there was actually a rise in the sedimentation rate after the course of treatment. These anomalies, together with the fact that in few of the cured cases

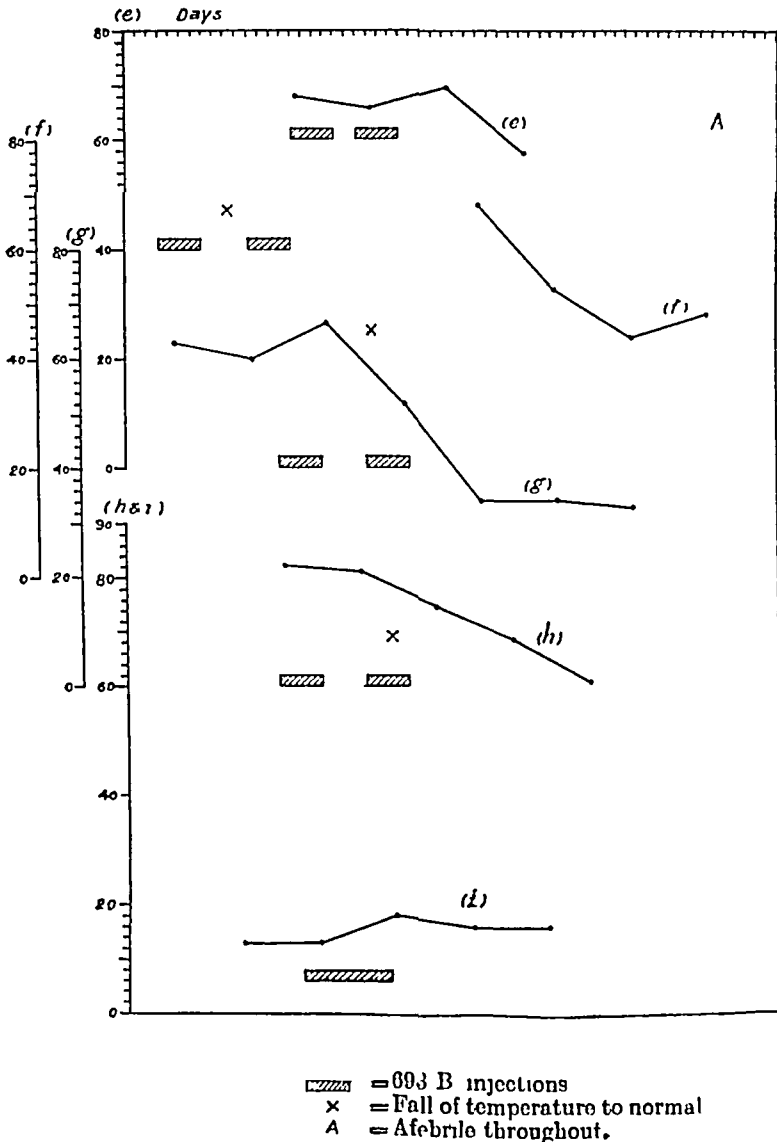
CHART 2
Good clinical progress



was there any marked drop in the sedimentation rate during the period of observation—which in most cases was longer than it is normally convenient to keep patients in hospital—lead one to the conclusion that this test is unlikely to provide a criterion of any prognostic significance.

Repeated estimations during the course of treatment — We shall consider first the cases in which the clinical progress was good and eventual cure without further treatment occurred (Charts 2 and 3), as the estimations were done once weekly it was not possible to say exactly at what point the sedimentation curve commenced

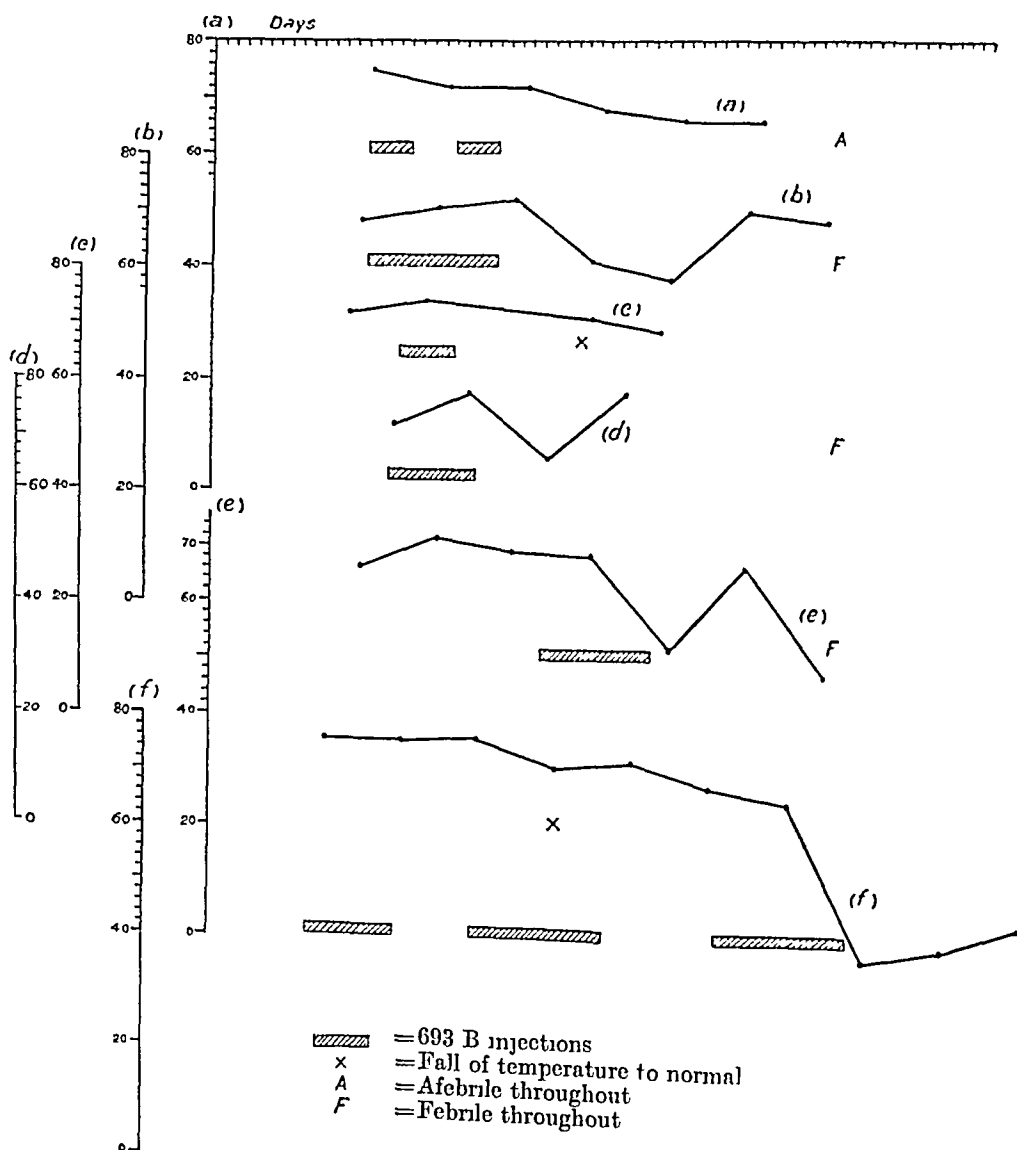
CHART 3

Good clinical progress

its downward tendency, but in 4 instances (*a*, *d*, *g* and *h*) this was obviously during the course of treatment, whereas in three instances (*c*, *e* and *f*) the fall was delayed for some little time after the treatment had been completed, in one instance (*b*) the

downward tendency was only slight and it was not clear when it commenced, and in the last instance (f) the sedimentation rate, which was normal before treatment, showed a tendency to increase

CHART 4

Unsatisfactory clinical progress or relapse

In the other group (Chart 4), the downward tendency of the curve was either only very slight [(a) and (c)], or the initial fall was rapidly followed by a rise [(b), (d) and (e)]. In the case of (f) of Chart 4, a patient who had relapsed repeatedly before, it was not until the third course of treatment was being given that there

was any appreciable fall in the sedimentation-rate curve, and even then it again took an upward turn

Although there is a distinct difference between these two groups of curves, as groups, there is no constant feature in each individual curve which would enable one to place it definitely in one group or in the other, compare, for example, Case (b) of Chart 2 with Case (c) of Chart 4, or Case (f) of Chart 3 with Case (f) of Chart 4. Therefore, no evidence of prognostic significance is likely to be obtained by carrying out a series of estimations in a patient under treatment

Sedimentation rate in relation to the 'aldehyde' reaction—Of the above cases, 13 out of 16 gave a definitely positive aldehyde result, and the other 3 a (+) reaction, a result which is classed as 'doubtful, probably kala-azar', in one of these three patients the sedimentation index was 63.00, that is, well below the mean for kala-azar patients, in another [Chart 1, Case (c)], 54.40, falling to 24.70 without antimony treatment and without any change occurring in the aldehyde reaction (this was the congenitally-syphilitic child referred to above), and in the third, 13.00, which is just about the normal mean. It is thus obvious that there is some association between the aldehyde reaction and the sedimentation time, but these few observations indicate that the latter does not give more reliable diagnostic information than the former. Therefore, as the aldehyde test is much more easily performed, we do not think that its displacement by the sedimentation-rate estimation for routine diagnosis need be considered

RELATIONSHIP OF THE SEDIMENTATION RATE TO THE TEMPERATURE OF THE PATIENT

In three instances the patients were afebrile throughout the course of treatment, in these nearly all the sedimentation estimations were high, 12 out of 16 registering over 60. In three other instances the patient was febrile throughout; in two of these the sedimentation index fell temporarily below 60. Of the remainder, in 4 instances the fall of temperature was coincident with the fall in the sedimentation rate, in 4 the fall in the former was not accompanied by any marked fall in the latter, and in one, afebrile at the beginning of treatment, the onset of a febrile reaction was accompanied by a slight rise in sedimentation rate. Fever and a high sedimentation rate both occur in kala-azar, and both tend to disappear with treatment, it is, therefore, natural that there should be some association between the two. However, the association is certainly insufficient to warrant the assumption that they are interdependent

SUMMARY

Estimations of the erythrocyte-sedimentation rate in normal persons and in kala-azar patients have been carried out by a previously-devised method, this method is described

The sedimentation indices of 28 apparently-healthy controls have a mean of 13 455, and a standard deviation of 7 101

The sedimentation indices of 16 untreated kala-azar patients have a mean of 67 675 and a standard deviation of 15 665, or, excluding one abnormally low reading of 13 00, the figures are 71 320 and 7 509, respectively

In cases in which the estimation was made several times at weekly intervals in the same patient prior to treatment being administered, the results were very constant

The means of the sedimentation indices in the cases in which progress was favourable and in those in which it was not favourable were practically the same

A more marked fall in the sedimentation index after treatment was noted in the cases in which progress was favourable than in the others, but the difference was not great

There appears to be some association between the sedimentation rate and the degree of intensity of the aldehyde reaction in kala-azar

There appears to be no association between the temperature of the patient and the sedimentation rate

CONCLUSIONS

Though the sedimentation rate is probably greater in kala-azar than in any other disease, it is concluded that the estimation of this rate is unlikely to prove a measure of any practical diagnostic value

It is concluded that no information of any prognostic significance will be obtained by carrying out either a single estimation, or a series of estimations in a kala-azar patient under treatment

EXPERIMENTAL OBSERVATIONS ON CHOLERA 'PHAGE LYSATE AS A COMPONENT OF PROPHYLACTIC CHOLERA VACCINE

BY

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[Received for publication, July 2, 1931]

D'HERELLE and his co-workers (1930) suggested that a strong and lasting immunity against cholera in man could be conferred by a single injection of 1 c c 'phage lysate of the corresponding vibrio. This statement is probably based on reported protection against 'Barbone' induced in buffaloes by injections of the 'phage lysate of *B. bovissepticus*. The type of immunity produced by injections of cholera 'phage lysate should be either antitoxic or antibacterial or a combination of both. As the bulk of bacterial bodies in a lytic filtrate undergo complete dissolution one would naturally expect to find in it more of the disintegration products of organisms (the so-called endotoxins of vibrio in this case) than intact bacteria themselves, in addition to bacteriophage protobes which grow at the expense of these organisms. Before attributing any property of inducing active antitoxic immunity to such filtrates it must be proved that they contain potent toxins as weak toxins can never act as satisfactory antigens (Kolmer, 1925).

In order to verify the toxicity of a virulent cholera 'phage lysate we prepared a filtrate containing the lysed products of the maximum number of cholera vibrio in a constant volume. This was obtained by inoculating varying amounts of a young culture of cholera vibrio to a series of broth tubes and a drop of virulent cholera 'phage added to each of these tubes.

TABLE

Showing the bactericidal and agglutination titre of sera of the two series of experimental rabbits

Rabbit No	Number of colonies developing on plates at 37°C after 24 hours' incubation with various dilutions of sera								Saline control	Complement control of normal rabbit serum	Agglutination titre
	1 in 200	1 in 400	1 in 800	1 in 1,600	1 in 3,200	1 in 6,400	1 in 12,800				
A series No 1 Wt 1,800 g	0	3	0	14	160	U C	U C	U C	U C	1/320 +++	
B series No 7 Wt 1,750 g	6	0	20	313	over 500	"	"	"	"	1/5,000 +++	
A series No 2 Wt 1,553 g	5	6	3	11	2	293	"	"	"	1/640 +++	
B series No 8 Wt 1,470 g	4	1	2	215	over 500	U C	"	"	"	1/640 +++	
A series No 3 Wt 1,533 g	8	3	8	23	47	40	"	"	"	1/640 +++	
B series No 12 Wt 1,400 g	4	2	1	20	23	70	"	"	"	1/1,280 +++	
A series No 4 Wt 1,400 g	0	1	17	23	97	206	"	"	"	1/320 +++	
B series No 9 Wt 1,600 g	1	11	18	182	227	U C	"	"	"	1/640 +++	
A series No 5 Wt 1,800 g	3	5	24	94	152	"	"	"	"	1/1,280 +++	
B series No 10 Wt 1,670 g	4	7	48	109	200	"	"	"	"	1/1,280 +++	
A series No 6 Wt 1,720 g	2	5	9	23	43	"	"	"	"	1/1,280 +++	
B series No 11 Wt 1,820 g	0	1	17	40	46	"	"	"	"	1/5,000 +++	

Note —U C = Uncountable

The tube receiving the maximum amount of cholera culture and at the same time showing complete clearance after 24 hours was used as the 'phage lysate for toxicity and immunity tests. 1 to 3 c.c. of 'phage lysate raised by the above method was injected subcutaneously in a series of 3 rabbits approximately of the same weight, while 3 similar rabbits receiving no injections were kept as controls. All the animals were carefully observed for two weeks and none of the injected animals behaved in any way differently from the healthy controls. After 15 days all 6 rabbits were given intravenously 16,400 millions each of killed cholera vibrio equivalent to $1\frac{1}{2}$ times the calculated minimum lethal dose per rabbit. All of them developed diarrhoea, collapsed and died in 24 hours. From the foregoing observations it can be safely concluded that the phage lysate of cholera vibrio in 1 to 3 c.c. doses subcutaneously was innocuous to rabbits and used as an antigen it could not protect them against $1\frac{1}{2}$ times the minimal lethal doses of dead cholera vibrio given by the intravenous route.

It was then considered that a combination of the homologous 'phage lysate with the standard cholera vaccine might be of distinct advantage as a prophylactic agent, as stimulation of opsonins, antibacterial, and antitoxic principles (d'Herelle 1926) has been attributed to 'phage lysates used as antigens. To test these two series of rabbits each containing 6 animals were selected. The 'A' series received each subcutaneously $\frac{1}{2}$ c.c. and 10 days later 1 c.c. of standard cholera vaccine containing 8,000 millions of killed vibrio per c.c. The 'B' series received each subcutaneously $\frac{1}{2}$ c.c. of standard cholera vaccine plus 1 c.c. of corresponding 'phage lysate and 10 days later 1 c.c. of cholera vaccine plus 1 c.c. of 'phage lysate. The animals of both series were bled 2 weeks after the second inoculation and their blood sera tested for agglutinogenic response by Harvey's technique (Harvey, 1920) and for bactericidal response by Hans Zinsser's method (Hans Zinsser, 1925). The result of observation has been summarized in the opposite table.

SUMMARY

(1) The 'phage lysate of cholera vibrio in 1 to 3 c.c. doses subcutaneously is innocuous to rabbits.

(2) Used subcutaneously as an antigen the 'phage lysate of cholera vibrio after a fortnight does not afford any protection to rabbits against 15 times minimal lethal doses of cholera vaccine given by the intravenous route.

(3) The addition of corresponding 'phage lysate to the standard cholera vaccine does not seem to enhance the bactericidal power of immune sera of experimental rabbits.

(4) Agglutination titres of immune cholera sera raised under the above experimental conditions are not always proportional to the bactericidal efficiency of those sera *in vitro*.

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THE PENTAVALENT COMPOUNDS OF ANTIMONY IN THE TREATMENT OF KALA-AZAR

Part VI.

A COMPARISON OF RESULTS WITH DIFFERENT COMPOUNDS

CORRIGENDA

In paper entitled 'A Note on the Expectation of the Relative Prevalence of Plasmodial Species when this is based solely on the Relative Output of Gametocytes' by Lieut Colonel H. H. King, M.S., in the October 1931 (Vol XIX, No 2) number of the *Indian Journal of Medical Research*, on page 354 the following corrections should be inserted —

3rd line of the first table last column for y^z read yz

In the second and third lines of the second group of tabulated figures

$$\text{for } x = n \text{ read } x = \frac{n}{2}$$

In the third line of the third group of tabulated figures

$$\text{for } \frac{n}{1} - 1 \text{ read } \frac{n}{2} - 1$$

In the last table last column the last set of figures, $\left(\frac{n}{3} - 1\right)$ etc are powers, not factors

Of the patients who were treated in each instance the series was a comparatively large one, the numbers were 104, 61, 52, 70, and 57, respectively. The treatment was carried out between July 1923 and July 1926 as the various drugs became available.

CLASSIFICATION OF RESULT

In analysing the results of treatment in an acute disease, such as pneumonia, the cases can be divided into those in which the treatment failed and death occurred, and those in which treatment was successful and the patient recovered, but in the treatment of kala-azar four things may happen: the patient may (i) be cured and subsequently remain free from the disease, (ii) undergo apparent cure and relapse later, (iii) show no improvement at all and be discharged in the same state as he was admitted, or (iv) he may die during the course of treatment. It is

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THE PENTAVALENT COMPOUNDS OF ANTIMONY IN THE TREATMENT OF KALA-AZAR

Part VI.

A COMPARISON OF RESULTS WITH DIFFERENT COMPOUNDS

BY

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[Received for publication, June 29, 1931]

INTRODUCTION

IN the earlier papers in this series the results of treatment by the more important pentavalent antimony compounds that are used in kala-azar have been analysed. The cases in these five series were unselected patients under hospital control, they were all treated under identical conditions, the diagnosis was in every case made by demonstrating the parasite, the temperature and weight records were accurately kept, on discharge a blood count and spleen or liver puncture was done, and in a very large percentage of cases the subsequent history of the patient was ascertained. In each instance the series was a comparatively large one, the numbers were 104, 61, 52, 70, and 57, respectively. The treatment was carried out between July 1923 and July 1926 as the various drugs became available.

CLASSIFICATION OF RESULT

In analysing the results of treatment in an acute disease, such as pneumonia, the cases can be divided into those in which the treatment failed and death occurred, and those in which treatment was successful and the patient recovered, but in the treatment of kala-azar four things may happen: the patient may (i) be cured and subsequently remain free from the disease, (ii) undergo apparent cure and relapse later, (iii) show no improvement at all and be discharged in the same state as he was admitted, or (iv) he may die during the course of treatment. It is

permissible to group the second and third class of results together, but—in the writer's opinion—the deaths should be considered separately

When the antimony tartrates only were used in the treatment of the disease we were inclined to think that deaths were inevitable and not in any way connected with the drug used, but more recent experience has changed our opinion on this point, deaths during treatment by the more satisfactory pentavalent compounds are rare, averaging less than 5 per cent, against 12 per cent to 25 per cent and even higher with the tartrates. Had the patients received no treatment probably 90 per cent would eventually have died, but it is doubtful if the death-rate within the period of two months—the time normally taken for the full course of antimony tartrate treatment—would have reached even 12 per cent, to quote the lowest figure. It is, therefore, almost certain that in the case of this drug death was hurried by treatment.

On the other hand, the fact that the same drug was administered in even larger doses to a very large number of persons suffering from oriental sore and from filariasis without causing deaths shows that the deaths were not due to the action of the drug on normal tissues, it is, therefore, apparent that the deaths occurring in cases of kala-azar in which the tartrates were given were due to their action on organs already extensively damaged by the disease. In the case of the more satisfactory pentavalent compounds it is apparent that the drug has very little adverse effect, even on the damaged organs. There is, for example, no evidence that there is any relationship between the amount of drug given and the death-rate, and the writer has shown elsewhere that huge doses of one compound may be given within a very short period without causing any ill effects clinically. Furthermore, in no instance in this series did the patient die immediately after the injections and only in one within 24 hours of an injection.

In this series the death-rate varied according to the drug used, so that it is a point which must be taken into consideration when the suitability of a drug is being appraised. However, the absence of any direct association between the amount of drug given and the death-rate suggests that the deaths which happened to occur after a particular total dose of the drug had been administered should not be associated with that particular dosage and added to the relapse for that particular dosage, but should be considered as a constant for the particular drug and be added to the relapse-rate for each particular total dose of that drug, when the probable recovery rate for any particular total dose is being calculated. For example, if for a drug 'x' the death-rate is 10 per cent, the relapse-rate 10 per cent for 5 grammes and 20 per cent for 3 grammes, the recovery rate for 5 grammes and 3 grammes would be, respectively, 81 per cent (i.e., 100 less 10 deaths less 9 relapses) and 72 per cent (i.e., 100 less 10 deaths less 18 relapses). Therefore, in all the analyses of the results obtained with the five different compounds the death-rate and relapse-rate have been considered separately.

THE DEATH-RATE

Some idea of the safety with which a drug can be administered is obtained from relative toxicity experiments with animals. However, the safety of a drug for administration to kala-azar patients is not always in proportion to its minimum lethal dose in mice. For example, in mice the toxicity of Stibosan and Bayer 693 are very much the same, the former being slightly more toxic, yet in a series of 61 treated by the latter none died.

Of the five series of cases the death-rate during treatment was as follows —

TABLE I

	Number in series	Number dying	Death rate to nearest figure per cent
1 Stibosan	104	11	11
2 Bayer 693	61	0	
3 Aminostiburea	52	2	4
4 Urea stibamine	70	4	5
5 Stibamine glucoside	57	2	4
TOTAL	344	19	6

From this table it is apparent that Stibosan is certainly the most toxic drug, Bayer 693 probably the least toxic, whereas the other drugs are about equal and come somewhere between these two extremes.

WHAT CONSTITUTES A CURE ?

The question then arises 'what constitutes a cure' ? The matter has been discussed at some length in previous papers of this series (Napier, 1926, 1927, 1928, 1929, 1929a) and elsewhere (Napier, 1924, and Napier and Halder, 1927). The usual criterion of cure in a parasitic disease is the disappearance of the causative parasite from the system, or the 'sterilization' of the patient. The difficulty arises in the demonstration of the presence or otherwise of the parasite. In diagnosing

the disease in an untreated patient there is little difficulty, the parasites are numerous and can be demonstrated, if not by direct microscopic methods, at any rate by cultural methods in at least 99 per cent of cases. But during and after treatment they disappear, first from the peripheral blood and then from the spleen and liver. A 'negative' blood culture has no significance whatsoever as the writer and others (Shortt, Das and Chiranjil Lal, 1927) have demonstrated. Experience shows that a 'negative' spleen puncture culture is considerably more significant, but the fact that parasites are not usually demonstrable in the spleen and yet abound in the skin in that interesting condition, post-kala-azar dermal leishmaniasis, is proof that absence from the spleen does not mean that there are none in the body. Furthermore, we have from time to time failed to demonstrate parasites in a spleen puncture culture after treatment in a patient who has subsequently relapsed. On the other hand, at the conclusion of treatment we have frequently demonstrated parasites in the spleen material of patients who have subsequently become 'sterilized' (as far as it has been possible to ascertain by laboratory methods) without further specific treatment. Apparently, the parasites are destroyed slowly by the body reactions originally stimulated by antimony injections, and are not destroyed directly by the action of the drug, a great percentage of which is excreted within the first 24 hours of administration (Boyd and Roy, 1929). Nevertheless, it does seem probable that the *rate* of the disappearance of the parasite from the spleen could be used as a measure of the efficacy of a compound. For this purpose, however, it would be essential that the same doses and time intervals should be observed. The importance of the time factor is shown by the fact that at the end of a course of 30 injections of sodium antimony tetrathionate, comprising about 2 grammes the liver puncture culture material is almost always sterile (Knowles, 1920), whereas after 2.3 grammes of Neostibosan administered in the 8 days parasites are almost always present (Napier and Mullick, 1928 and 1929), yet the latter is a more efficient course. In the four series under discussion neither the time nor the dosage have been constant so that this comparison cannot be made.

The parasitological method of predicting a cure having proved fallacious on both the positive and the negative side, other methods must be considered. As the serum's return to normal is considerably delayed, taking at least 4 months in advanced cases, no serum tests done at the time of discharge are of any value, and the preparation of a protein graph would be too laborious for practical purposes, even if it were found to give reliable information.

The clinical progress of the patient under treatment cannot be considered as a reliable criterion of cure, although some indications can be obtained and for this purpose the various factors in the clinical progress with the different drugs might be compared, this will be done later. Meanwhile, we are thrown back on the subsequent history of the patient as the only reliable method of judging whether

a cure has occurred or not. There again we are up against two difficulties, the question of the time the patient should be kept under observation and the possibility that re-infection may sometimes occur.

RELAPSE OR RE-INFECTION?

We have looked up the notes of a number of patients who have apparently relapsed after treatment either elsewhere or in this institution. In a few instances the notes were incomplete, but in 51 the time which elapsed between the conclusion of treatment and the re-appearance of the symptoms was noted. The figures obtained have been tabulated below and a graph has been drawn —

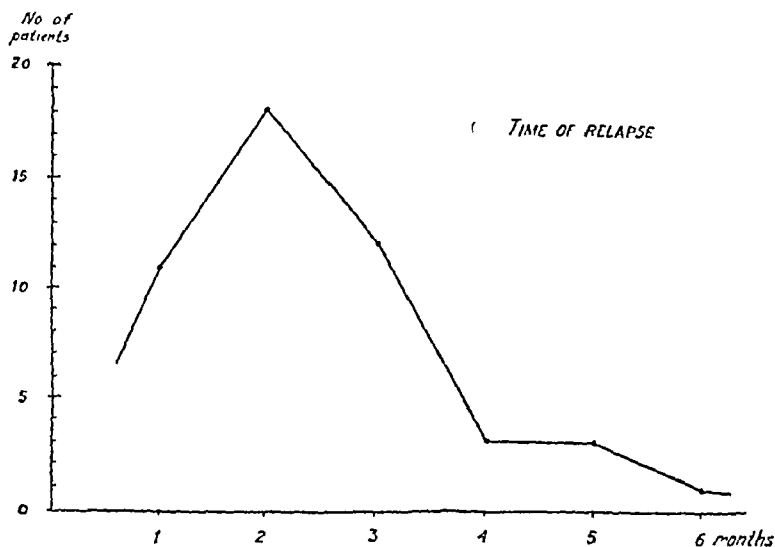
TABLE II

Period elapsed after treatment before re appearance of symptoms	Number of cases
1 month, or less	11
About 2 months	18
„ 3 „	12
„ 4 „	3
„ 5 „	3
6 „	1
„ 1 year	3
TOTAL	51

The Graph gives almost complete answers to the two questions. It shows, firstly, that if the patients are kept under observation for 6 or 12 months practically all relapses will be recorded, and, secondly, that the very large number of the cases classed as 'relapses' are real relapses and not re-infections. Of the 3 patients in whom symptoms re-appeared after about one year, 2 of them had been in comparatively good health with occasional mild symptoms at the end

of a year their spleens were very large and hard, and their aldehyde reactions were 'strongly positive', so it is fairly certain that they had been suffering from a chronic form of the disease for a considerable time. The other patient, who had originally been treated within a month of the onset of the symptoms of the first attack and had returned to her home in the endemic area, returned exactly one year later with a clinically identical attack, the aldehyde and antimony tests were again entirely negative. This appears to have been a case of re-infection, it is the only instance in our experience in which re-infection is strongly suggested. The rarity of re-infection suggests that in the majority of cases some immunity is established, and this case suggests that if the patient is treated in the early stages immunity may not be established.

GRAPH



Showing the period which elapsed between the conclusion of treatment and the reappearance of symptoms in 51 cases in which a 'relapse' occurred, in 3 patients the period was between 6 months and one year.

It is thus apparent that a period of good health for six months can be looked upon as an indication that the patient is cured. This appears to be the only reliable criterion and it is one that we have adopted throughout this series.

THE RELATIVE EFFICACY OF THE VARIOUS ANTIMONY PREPARATIONS

The difference between the antimony tartarates and the pentavalent antimony compounds in the treatment of kala-azar is so striking that it does not require more than a small series of cases treated by each class of drug for its demonstration. When, however, one comes to differentiating between the various pentavalent compounds the case is very different. For this purpose reference to the ordinary

hospital or dispensary records of treatment are quite valueless, not only because the diagnosis is seldom confirmed by demonstrating of the parasite, but because different physicians adopt not only different schemes of dosage, but different standards for judging whether a patient is cured or not. During a recent visit in Assam the writer found that in some of the Government dispensaries the average number of injections given to each patient, if he continued to attend sufficiently long, was about 18. The drug used was urea stibamine. In our series (No. IV) it will be seen that by giving a course of 10 injections highly satisfactory results were obtained, but an analysis of data from the Assam dispensaries might lead one to suppose that a much more extensive course than this was advisable.

Even when the treatment is given by one observer who controls both diagnosis and cure scientifically, difficulties will arise. The only satisfactory methods of comparing the efficacy of two drugs is by preparing cure-rate curves.

THE ADOPTION OF A STANDARD COURSE OF TREATMENT

The present writer analysed a series of cases in which the antimony tartrates were used, he found that all the relapses occurred amongst the patients who had received the largest total doses. This anomalous state of affairs is easy to explain. Partially 'resistant' patients usually improve very slowly, so that there is a tendency to give them longer courses of treatment, but despite this relapse is more likely to occur in this class of patient than in the ordinary patient. From such records it is impossible to construct a relapse-rate curve, as there is a non-measurable factor, the judgment of the physician, to be taken into consideration. For the figures to be of any value either the dosage must be entirely random and totally uninfluenced by the progress of the patient, or a number of dosages must be decided upon and each dosage applied in a certain number of unselected cases, again without reference to the severity of the case, or to the rate of progress under treatment. There are certain considerations which make the random dosage plan unsuitable for adoption amongst hospital patients. Out-patients are liable to discontinue treatment for reasons unconnected with their rate of progress towards cure, the writer (Napier and Halder, 1927), taking advantage of this fact, analysed a series of cases treated by sodium antimony tartrate and was able to obtain figures which made fairly smooth cure-rate curves for this drug.

The method of adopting a definite dosage to be applied irrespective of the progress of the patient also presents certain difficulties. A considerable amount of preliminary experimentation in dosage is necessary as it would be unfair to subject a large number of patients to a totally insufficient dosage. In most of the 5 series referred to above, the preliminary experimental stage was scarcely passed, but in some of the later cases of the series an attempt was made to give a fixed course, for example, in Series II (Bayer 693) 19 previously-untreated patients were given a course of 10 injections. Consequently, an attempt was made to draw

a cure-rate curve in only one series (No I, Stibosan) and as the plotted points are based on a very small number of observations even that curve cannot be looked upon as accurate. For the rest we have been content to indicate the cure-rate for certain dosages.

DIFFERENT METHODS OF EXPRESSING DOSAGE

There are at least three different ways in which the dosage may be expressed, by the number of injections, by the actual total dose, and the total dose relative to the weight of the patient. Where different individual doses are given and where patients of different ages are treated contradictory results are frequently obtained. For example, in order to produce a certain cure-rate, the total dosage of sodium antimony tartrate which is required is very much the same as that of Stibosan, yet the number of injections that are required is three times as great in the case of former as in the case of the latter drug. Even when the required total dosages are about equal, the number of injections is another point of great practical importance to be considered, it is obviously advantageous to use a less toxic drug with which the total dosage can be given in 5 injections in 5 days than one which necessitates division into 30 injections spread over a period of 2 months.

EXCLUSION OF PREVIOUSLY RELAPSED CASES

For all these calculations only patients who have previously received no treatment must be included. It is obvious that recent previous treatment might vitiate the results, and on the other hand a patient who has received a full course of treatment and has relapsed is a 'resistant case', not necessarily because treatment has made him 'resistant', or 'antimony-fast', but because the fact that he failed to react to an ordinary course of treatment has shown that he is a 'resistant case'. Any fair sample of the kala-azar population may contain 'resistant cases', but a sample which contains *known* 'resistant' cases is not a fair sample, such cases have been selected from the general kala-azar population by a process of sifting, although the sifting may have been done elsewhere.

OTHER METHODS OF COMPARISON

As it has not been possible to draw cure-rate curves for these drugs other methods of judging relatively the value of different drugs will have to be considered. Two methods which suggest themselves are (a) comparison of the mean actual total doses, relative total doses or the number of injections which produced a cure, and (b) comparison of the percentage cure rate amongst all patients given not more than some definite dose—as for example, 3 grammes actual total dose, or 4 grammes relative total dose—or some definite number of injections.

This has been done with the five drugs under comparison, the figures are summarized in the table below —

TABLE III

Drug series	Total number receiving not more than 3.0 grammes total actual dose		Total number receiving not more than 4.0 grammes total relative doses		Number receiving not more than 10 injections		Mean of total actual dose of cured patients	Mean of total relative dose of cured patients	Mean of number of injections given to cured patients
	Cases	Relapses	Cases	Relapses	Cases	Relapses			
I	39	4	34	4			2.68	3.88	(13.4)
II	37	0	35	0	26	0	2.06	3.31	10.34
III	31	(2)*	21	(2)*	10	(1)*	2.23	3.30	11.37
IV	45	1	42	1	18	0	2.19	3.00	11.04
V	23	5	32	6	24	3	2.16	3.18	11.21

* Previously reported as not traced

Discussion — In the five series the mean actual and relative total dose and the mean of the number of injections were greatest in Series I (Stibosan). In the other four series the figures are about equal. Whether the actual or the relative dose, or the number of injections be considered, the figures indicate a lower curative power in Series I (Stibosan) and V (Neostam), and, although the actual figures indicate No. II (Bayer 693) as having the highest curative power, the number of relapses is so small that on these grounds alone it would be unfair to be dogmatic.

THE RATE OF CLINICAL PROGRESS

Although the final result, that is cure or failure to cure, is the important point, the rapidity of the progress of the patient under treatment is also important. This can be expressed under four headings —

- (a) The time of the cessation of fever
- (b) The reduction in the size of the spleen
- (c) The increase in weight
- (d) The white-blood-cell count

(a) The number of injections administered prior to the cessation of fever is shown in the following table —

TABLE IV

Series	Number of patients	Number afebrile throughout	Number febrile throughout	Mean number of injections of the rest
I	77	3	4	5.7
II	58	3	1	4.57
III	48	2	0	5.3
IV	64	5	4	4.93
V	53	3	6	4.74

The reason for separating the cases which show fever throughout is that there appears to be a certain type of case in which the fever is actually kept up by the injections, falling directly they are discontinued. Here No. II is again the most favourable. After this there is little to choose between Nos. III, IV, and V, in No. III the mean number of injections given is higher than the others, but in 4 and 6 patients, respectively, in Series IV and V the temperature remained high throughout.

(b) The size of the spleen at the time of discharge is shown in the following table —

TABLE V

Showing size of spleen at time of discharge

Series	Number of patients	Number in which spleen was not palpable	Number in which spleen was palpable, but not measurable	Number in which spleen was measurably increased	Average size of spleen in cases of previous column
I	74	26	26	22	3.0
II	56	21	28	7	2.7
III	48	11	28	9	2.61
IV	62	17	33	12	2.7
V	52	18	21	13	2.7

There is again not much to choose between the different compounds, No I would appear to be the least and No II the most satisfactory

(c) The weight increase or loss is shown in the following table —

TABLE VI

Showing change in weight during treatment

Series	Number of patients	Number increasing in weight	Number showing no change	Number losing weight	Mean increase in lb
I	77	69	5	6	6.91
II	57	52		5	6.72
III	48	16	0	2	7.00
IV	61	52	2	7	5.55
V	52	46	3	3	6.85

In this table No III shows the best results and No IV the least satisfactory, however, the difference between these two series is not very striking

(d) The means of the total white-blood-cell count at the time of discharge is given in Table VII —

TABLE VII

Series	Number of cases	Means of the total white blood cell per c mm
I	77	7,654
II	53	7,545
III	42	6,788
IV	56	7,851
V	45	7,107

DISCUSSION

Judging the efficacy of these five drugs on the rate of progress of the patients during treatment, it is not possible to say that any one gave strikingly better results than the rest, throughout, however, No II gave satisfactory results, probably the most satisfactory, and No I the least satisfactory.

COMPARABILITY OF THE RESULTS

It is notorious that in certain infections, cholera for example, there is a considerable falling off in the severity of the disease towards the end of an epidemic, it has been suggested that the great success in the treatment of kala-azar by the new antimony compounds might be partly due to this factor. It is true that the advanced cases with cancrum oris, which one saw comparatively frequently ten years ago, are now rare. The reason for this is, in the writer's opinion, not a decrease in the severity of the disease, but the great advance that has been made during recent years, both in the treatment and in organization by which the people in remote districts are enabled to get the treatment.

Until very recently we have given sodium antimony tartrate as the standard treatment in our out-door department, and we did not notice that these patients progressed any more rapidly than they did a few years previously.

The disease has been endemic in Bengal for a great many years. Periodic exacerbations of the disease do occur, and there is little doubt that we are now in the trough of the wave, but there is no evidence to show that these variations in the incidence are accompanied by corresponding variations in the intensity of the disease in the individual. The treatment in each of the series was carried out, though not at the same time, under exactly the same conditions on the same class of patient, and are, therefore, strictly comparable.

DISCUSSION

A review of the evidence before us will show that No. I may be excluded on the grounds that the highest death-rate followed its use, and the cure-rate with both 3 and 4 grammes was poor, whereas No. V can be excluded on account of the very poor cure-rate. Amongst the rest there is little to choose, but throughout No. II appears to show a slight superiority. With regard to No. III in a subsequent series, in which treatment was carried out elsewhere under the writer's supervision, the results were less satisfactory, and there appears to be some doubt whether either No. III or No. IV are constant chemical compounds, it has been suggested that the latter is more of the nature of a mixture and, therefore, very liable to variations in content. Ghosh, Chopra and Chatterjee (1928) have recently pointed out that commercial samples may show considerable variations. Furthermore, Bayer 693 is a compound with a high antimony content and a low relative toxicity, so that, if occasion arises, a much larger individual dose can be given safely than has been given in any of the series reported. It was, therefore, decided that, for future investigations into the question of the most suitable amount, strength and spacing of the injections, and of optimum total dosage, one drug, namely Bayer 693 (No. II of this series), should be used.

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THE PENTAVALENT COMPOUNDS OF ANTIMONY IN THE TREATMENT OF KALA-AZAR

Part VII.

NEOSTIBOSAN DI-ETHYL-AMINE PARA-AMINO-PHENYL STIBIATE, 254 CASES

BY

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[Received for publication, June 29, 1931]

INTRODUCTION

IN a previous paper, No II of this series, the results of treatment in 61 cases of kala-azar by means of Bayer 693,* di-ethyl-amine para-amino-phenyl stibiate, were recorded and analysed. For various reasons, stated in another paper, No VI of the series, this drug has been chosen for further investigation. Another consignment of Bayer 693 was obtained at the end of 1926 and at the beginning of 1927 a further series of 33 patients were treated, the present writer then went on leave. During the time he was in Europe he visited the factory of the I G Company at Leverkusen and Elberfeld and had a number of discussions with Professor Schmidt of the Research Department of this company. The only defect that he had noted in Bayer 693 was that in a small percentage of cases it caused vomiting, this point was discussed with Professor Schmidt and as a consequence he modified his method of preparing the compound. The new product which had the same chemical formula was known as Bayer 693B. Professor Schmidt reported that by intramuscular injection it was much less toxic to mice than Bayer 693,

* This preparation has been referred to elsewhere as von Heyden No 693, but for some years the antimony work of the firm of Heyden has been taken over by the I G Company and the preparation is now known as Bayer 693.

and in use in human beings we found that it very rarely caused vomiting even when given in large doses. By intravenous injection in mice we did not, however, find it any less toxic than Bayer 693, the results of the toxicity experiments are given below —

Dose of 693B in milligrams per kilo body-weight of mouse	Mice		
	Average weight of group in grammes	Number	Number surviving after 18 hours
200	20.8	5	5
250	22.2	5	3
300	21.6	5	2
400	21.4	5	2

After a short clinical trial with the compound, prepared by the new process, we were able to report that the therapeutic action was apparently very similar indeed to that of the original samples. Up to this point the manufacturers had withheld this compound for the general medical public and had only supplied it to the present writer for experimental purposes. Now, however, on his advice, it was placed on the market under the trade name 'Neostibosan'. That these two compounds, Bayer 693 and Bayer 693B, are not chemically identical is observed from the fact that the antimony content of the latter is slightly greater than that of the former, 42 per cent instead of 41 per cent, but the therapeutic action appears to be identical, this we will show below.

THE RESULTS OF TREATMENT WITH THIS PREPARATION

This series includes all the patients treated by Bayer 693 or 693B up to the end of the year 1928. The total number is 254.

The immediate results of treatment can be summarized as follows —

Discharges as cured	244
Failed to respond to treatment	4 or 1.57 per cent
Died during treatment	6 or 2.36 „

Of the 244 patients discharged as cured 27 have not been traced, the results of treatment, as judged by the clinical condition of the patient 6 months

TABLE I

Course of treatment	Number of cases	CURED		RELAPSED		FURTHER TRFATMFNT		DIED SUB-SEQUENTLY FROM OTHER CAUSE
		Primary	Resistant	Primary	Resistant	Primary	Resistant	Primary
No 693								
11 or more injections	21	15	6					.
10 injections spaced	28	24	3	1				..
8 injections spaced	30	28	.	2				..
No 693B								
More than 10 injections	2		1		1	.		.
10 injections spaced	4		3		1			
8 injections spaced	41	37		2		1		1
8 injections concentrated	74	59	4	5	1	1		4
5 injections concentrated	13	10	1			2		.
4 injections	2	1				1		
2 injections	2	1				1		
TOTALS	217	175	18	10	3	6		5

after discharge, of the remaining 217 is known (see Table I) and can be summarized as follows —

Completely cured	199
Died within 6 months of discharge from some other disease	5 or 2 30 per cent.
Relapsed	13 or 5 99 „

Thus, if we deduct the failure-rate, the death-during-treatment rate and the relapse-rate we have left a cure-rate of 90 08 per cent, or if in addition we deduct the death-from-other-causes rate we have a cure-rate of 87 78 per cent

The above figures are for all cases, including previously-treated cases, if we exclude the latter the cure-rate figures for primarily-treated patients are slightly improved. The 4 'failures' had all been treated previously and had relapsed, the other figures are —

Completely cured	181
Died within 6 months of some other disease	5 or 2.55 per cent
Relapsed	10 or 5.10 „

Thus, for primarily-treated cases there are no failures and by deducting the relapse-rate and the death-rate one gets a cure-rate of 92.54 per cent, or if in addition we deduct the death-from-other-causes rate we get a cure-rate of 89.99 per cent.

COMPARISON OF THE TWO DIFFERENT SAMPLES

The relapse-rate with Bayer 693 was only 3 in 79 cases, or 3.77 per cent, whereas with Bayer 693B it was 10 out of 138, or 7.24 per cent. If the resistant cases are excluded there is less disparity between the two sets of figures. The comparison is not however fair, as far bigger doses were given in the case of Bayer 693. A fairer comparison can be made by taking only the cases in which 8 injections were given on alternate days. In the case of Bayer 693 there were two relapses in 30 cases, with Bayer 693B in 41 cases there were two definite relapses and in a third case, a relapse being anticipated, a further course of injections was given, counting 3 relapses for the latter we have relapse-rates of 6.67 per cent and 7.32 per cent, respectively, with the two preparations, the difference here is negligible.

Taking into consideration one factor only in the rate of response to treatment, namely the rate of fall of temperature, the average number of injections given prior to the cessation of fever was 4.23 in the 30 cases treated by Bayer 693, and 4.59 in the 41 cases treated by Bayer 693B. Again the difference is slightly in favour of Bayer 693, but is not significant.

Conclusion — In view of the absence of any significant difference between the therapeutic results obtained with these two compounds, it seems justifiable to treat them as the same compound as far as their therapeutic properties are concerned, subsequently in this paper no distinction will be made between these two preparations.

DOSAGE IN RELATION TO CURE AND RELAPSE

As well as actual relapses all potential relapses, that is, cases in which further treatment was considered advisable after the full course had been given, are classed as relapses.

The mean total actual dose given to the 175 previously-untreated patients who were cured was 2.064 grammes and to the 16 classed as relapses 1.867 grammes.

The mean total relative dose of the cured cases was 3.01 grammes and of those classed as relapses 2.74 grammes

By dividing the patients into groups according to the total actual doses given the following figures are obtained —

TABLE II

Dosage	Total cases	Cures	Re lapses	Cure rate, per cent	Mean dose for these cure rates (in grammes)	
0.5 gramme or more but less than 1 gramme	12	11	1	91.67	85.7	1.04
1.0 , , 1.5 grammes	16	13	3	81.25		
1.5 grammes , , 2.0 ,	30	27	3	90.0		1.74
2.0 , , 2.5 ,	117	109	8	93.16		2.24
2.5 , , 3.0 ,	13	12	1	92.3	94.12	2.83
3.0 , , 3.5 ,	4	4	0	100.0		

By re-grouping them according to the total relative doses the following figures are obtained —

TABLE III

Dosage	Total cases	Cures	Re-lapses	Cure rate, per cent	Mean dose for these cure rates (in grammes)	
0.5 gramme or more but less than 1 gramme	2	1	1	(50)	1.38	
1.0 " " " 1.5 grammes	2	1	1	(50)		
1.5 grammes " " 2.0 "	4	3	1	(75)		
2.0 " " " 2.5 "	34	32	2	94.1	90.05	2.57
2.5 " " " 3.0 "	61	54	7	88.5		
3.0 " " " 3.5 "	51	49	2	96.1	95.5	3.64
3.5 " " " 4.0 "	15	15	0	(100.0)		
4.0 " " " 4.5 "	16	15	1	93.7		
4.5 " " " 5.0 "	4	4	0	(100.0)	(50.0)	
more than 5.0 grammes	2	1	1	(50.0)		

And lastly by grouping them according to the number of injections they received the following figures are obtained —

TABLE IV

Dosage	Total cases	Cures	Relapses	Cure rate, per cent
11 or more injections	15	15	0	(100 0)
10 injections	25	24	1	96
8 „	135	124	11	91 85
5 „	12	10	2	83 3
4 „	2	1	1	(50 0)
2 „	2	1	1	(50 0)

It is apparent that there is a definite relationship between the total dosage and the cure-rate, whether the actual dose or the dose relative to the weight of the patient be considered. The fact that the mean dose of the 'cured' cases was greater than that of the 'relapse' cases suggests that at least some of the patients of the latter group received less than the mean curative dose.

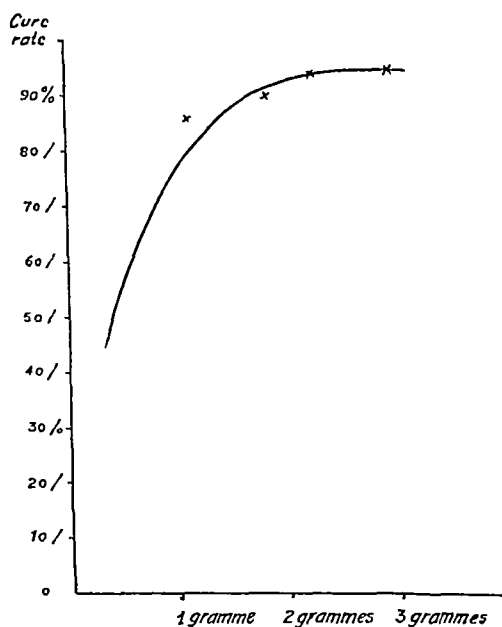
This relationship will be seen more clearly in the cure-rate tables. When the groups are arranged according to even ranges of dosage some of them are very small and irregularities in the cure-rate curve are inevitable, but these can be smoothed out by bunching a number of groups together. The relative dose cure-rate curve was distorted by two cases of young children in which despite a very large relative dose being given a relapse occurred, as far as adults are concerned there was not a relapse in any case in which a relative total dose of more than 3.5 grammes was given. This confirms the observation previously made that children require relatively larger doses than adults.

On the Graphs 1 and 2 a number of points have been plotted for the actual and relative cure-rates, respectively, at different dosage and free-hand curves, which approximate very closely to these plottings, have been drawn. It cannot be claimed that these curves are an accurate representation of the cure-rates, but it

seems probable that they indicate fairly closely the general direction that would be taken by these curves were it possible to obtain a series sufficiently large to eliminate errors of chance sampling. The tendency in both curves is the same, there is a rapid rise up to a certain point, after which they become flat and eventually they run almost horizontally. In the actual-dose curve the rise is scarcely appreciable after 2.25 grammes, or in the relative dose curve from about the 3.0 gramme point.

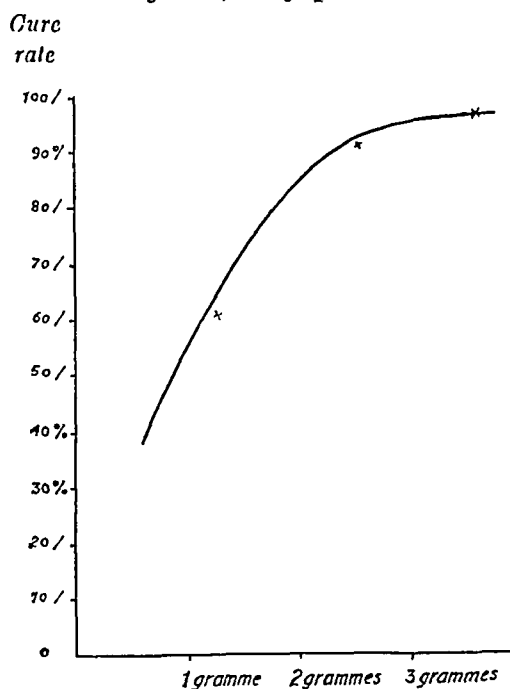
GRAPH 1

Cure-rate
Actual total dose



GRAPH 2

Cure-rate
Total dose per 100 lb
body-weight of patient



A table (Table IV) showing the cure-rates with different numbers of injections has been prepared, the number of injections was not necessarily proportionate to the total dose so that *per se* this table has little significance. This point is discussed later.

Conclusion—It would thus appear that where large numbers of patients are to be treated actual total doses of 2.25 grammes or relative doses of 3.0 grammes are the most suitable, to give more would be uneconomical and to give less would mean a distinct increase in the relapse-rate.

THE RELAPSES

Of this 16 cases classed as relapses, 6 were cases in which further treatment was given whilst the patient was still in hospital because the clinical condition of the patient appeared to be unrelieved by the first course of treatment, all of those were cured by the subsequent treatment. Of the 10 relapsing cases 2 died with typical symptoms of a relapse, 4 received further treatment and were cured, and 4 proved entirely resistant to further treatment. It seems probable that these 4 patients would have been resistant whatever course of treatment had been given and that they represent the constant percentage of unresponsive patients which are probably to be found in any series, in this series they constitute roughly 2 per cent of the total. A relapse percentage greater than this must be considered to be due to insufficient treatment.

THE METHODS OF ADMINISTRATIONS

The routine method usually employed has been the intravenous injection of a 5 per cent solution, but in a few cases a 25 per cent solution was used. There appeared to be no disadvantage in the injection of the more concentrated solution, as evidenced either by the immediate results or by the patient's rate of progress towards cure, but this solution has not been employed in a sufficiently large number of cases to allow of any separate consideration of its effect on the cure-rate.

Intramuscular injection of a 25 per cent solution was given in 10 cases. The ordinary dosage was adopted and it was found that up to 0.4 gramme it was tolerated well, in 9 instances daily injections were given and in the other injections were given on alternate days. The average time of the fall of temperature was the 10th day, as against a mean of 9.14 days for other cases in which 8 daily injections were given. None of the patients relapsed, one died within a few months of a condition which suggested cerebral malaria, and nine were completely cured.

Conclusion—The intravenous injection of a 5 per cent solution would appear to be the method of choice, but there appears to be no reason why a 25 per cent solution should not be given intravenously, or why, if difficulty arises in giving the injections intravenously, a 25 per cent solution should not be given intramuscularly.

DISTRIBUTION OF THE DOSAGE

It has been suggested above that a total actual dose of about 2.25 grammes is the optimum, there are a number of ways in which this dose can be divided up. In Table IV the cases are divided up according to the number of injections they received, and in Table I some of these groups have been further subdivided according to whether the patients received injections daily or on alternate days. When 8 or 5 injections were given the total dose was usually about 2.3 grammes, but when either 10 or more, or less than 5 injections were given the total dose was usually distinctly greater or distinctly less than 2.3 grammes. Thus, if we are to gauge the advantages

of the different methods of dividing the dosage, we must take only previously untreated cases of the groups in which adult patients received a total dosage of about 2.3 grammes, these are grouped as follows —

TABLE V

Group	Nature of course	Cured	Relapsed	Further treatment	Died subsequently
A	8 injections, on alternate days	65	4	1	1
B	8 injections, given daily	59	5 (less 1) *	1	4
C	5 injections, given daily	10		2	0

* Should possibly be excluded, see text

Of the 69 patients of Group B, 4 died during the 6 months' probation period of causes other than kala-azar. The proportion is so large that one cannot help wondering if this course of injections tends to reduce the patient's resistance to other infections to an unusual degree, however, there were no deaths reported in the 12 cases in which still more concentrated course was given.

With regard to actual relapses the figures for Groups A and B are practically equal, as in one relapsing case in Group B the patient had a very modified dosage on account of severe reactions following the first two injections and should possibly be excluded.

In Group C no cases of relapse occurred, but in two instances as the patients showed little clinical improvement after the first course of injections, further treatment was given at an unnecessarily early date, there was a misunderstanding of the writer's instructions, and in his opinion one of the patients, at least, did not require further injections.

The actual doses given in the case of the adults in this group was 0.3, 0.4, 0.5, 0.6, and 0.6 gramme. The first two patients who were given this course were selected as being suitable for experimental treatment, but subsequently all patients admitted during a certain period were given this course, some of these patients were definitely debilitated. No death occurred amongst these patients and in only one case was it thought advisable to modify the course, this was on account of a severe febrile reaction following a dose of 0.5 gramme.

The time factor should be taken into consideration. The course in which 10 injections were given usually occupied about 22 days, the injections being given thrice weekly, and the ordinary 8-injection course 15 to 18 days, the concentrated 8-injection course 8 days and the 5-injection course 5 days. The interval between the conclusion of treatment and the puncture was the same in each group, but if the

time is taken from the commencement of treatment it will be seen that the interval varies between 36 and 19 days according to the course given, it would appear that in every case the parasites have disappeared by the 36th day whereas as early as the 19th day they are still present in 44·4 per cent of cases. It was seldom possible to keep the patients in hospital for long periods after treatment has been completed, but in two instances circumstances allowed this. The first patient received 8 injections on alternate days, his spleen culture showed leishmania on the 28th day from the commencement of treatment but on the 56th day it was negative. The second patient received 8 injections on successive days, his spleen culture was positive in the 23rd day, but negative on the 46th. It is suggested that the commencement of the change in the body chemistry which leads to the disappearance of the parasites from the spleen and liver occurs at the time of the first injection, although in most cases its continuance is dependent on further injections being given.

It is obvious that the time factor is important, that the 14 days' interval between the last injection and the spleen puncture is inadequate in many instances, and that, if the spleen or liver puncture culture is to be accepted as evidence of cure, the culture should be taken from 5 to 6 weeks after the commencement of treatment.

Progress under treatment—Though the most important point is the actual results of treatment, yet it would seem reasonable to expect that some indication of the efficacy of a course of treatment could be obtained from a study of the progress of the patient under treatment, the points to be considered are cessation of fever, leucocyte count, increase in weight, and reduction in the size of the spleen.

(a) *Cessation of fever*—The behaviour under treatment of the cases of the various groups can be summarized as follows—

TABLE VI

Group	Nature of course	Cases in which fever fell to normal, and mean number of days of fever		Number afebrile throughout	Number febrile throughout	Average duration of treatment in days
		Number	Days			
	Previously reported series, average 10 injections or more	54	9·66	3	1	24
A	8 injections on alternate days	54	8·01	3	5	17·5
B	8 injections, daily	60	8·02	2	3	8
C	5 injections, daily	10	6·3		1	5

It will be seen that the concentration of the treatment does not cut short the febrile period to any great extent, there is a tendency in the concentrated courses for the fever to remain up just as long as the injections are continued

(b) *The leucocyte count* —For the counts done roughly two weeks after the last injection the means are much the same for each of the first three groups, namely, 7,545 per c mm for the cases previously reported (mostly 10 injections or more), 7,848 per c mm for Group A (8 injections on alternate days) and 7,341 per c mm for Group B (8 injections, daily), but for Group C, in which 2.3 or 2.4 grammes were given in 5 days, there is a decided increase to 9,449 leucocytes per c mm

(c) *Increase in weight* —For the four groups the mean of each series showed increases of 6.72, 5.56, 4.74 and 5.64 lb, respectively. Here it is apparent that time has played an important part, except in the last series, but it is probable that chance has affected this figure as the group is a small one

(d) *The size of the spleen* —This is best expressed in tabular form —

TABLE VII

Group	Nature of course	Not palpable	Palpable	MEASURABLY ENLARGED	
				Number	Average size in inches below costal margin
	Previous series, average 10 injections	21	28	7	2.71
A	8 injections, alternate days	25	26	6	2.50
B	8 injections, daily	19	39	8	2.75
C	5 injections, daily	2	8	1	2.50

Here again time appears to be the important factor, in Group A a much larger proportion of cases had spleens which were not palpable than had the cases of the other two groups in which the time under treatment was much shorter

To summarize, it appears that by giving the concentrated course of 5 injections the period of fever is shorter and the leucocyte increase is greater than with the other two courses, that the increase in weight is about the same as the average of the other two courses, and that though the reduction in the size of the spleen is less marked, this is probably on account of the shortening of the length of the time under treatment and therefore of the time the patient remains under observation. There is a possible fallacy in the fact that only a small number of patients, eleven, are included in this group

Conclusions —One must admit that the evidence of the question of distribution of dosage is not entirely conclusive, but that there would appear to be no disadvantage in giving injections daily

A study of the rate of progress of the patients under treatment suggests that the 5-injection course may prove the most satisfactory, but this point cannot be finally decided without further experience

CRITERIA OF CURE

The evidence of cure in this series is the same as that of all the previous series, either a clear, written statement by the patient himself, or his relative or medical adviser, or a personal examination of the patient, at least six months after discharge from hospital. In only one instance in his experience has the writer seen a relapse occur in a patient who has been reported cured after this interval, this did not occur in this series

Of the patients of Group A (Table VIII, *see* below), excluding the cases in which a relapse occurred, 11 were seen more than 6 months after discharge, of these one had shown leishmania at the time of discharge. Of the patients of Group B, 17 were seen, of these 4 had shown parasites at the time of discharge. Of the patients of Group C, 5 were seen, of these 2 had shown parasites at the time of discharge. Thus 7 patients in whom leishmania had been found at the time of discharge when seen 6 months or more later were clinically entirely free from symptoms and their aldehyde tests were negative. One patient had dermal leishmaniasis but this patient had no visceral infection. The complete evidence of cure in these cases shows that there is little reason to doubt the history in the remainder

The presence of parasites — In this connection it will be best to consider only primarily-treated cases. In nearly every case a spleen or liver puncture with culture was done at the time of discharge. The interval between the last injection and the puncture was usually the same, about a fortnight. The results of the successful cultures (contaminated cultures being excluded) in the various groups were as follows —

TABLE VIII

Group	Nature of course	CULTURE RESULTS			NUMBER OF CASES IN WHICH RELAPSES OCCURRED AMONGST CASES SHOWING	
		Negative	Positive	Per cent	Negative culture	Positive culture
	10 injections or more	30		100		
A	8 injections, alternate days	39	8	93.0	2	2
B	8 injections, daily	37	17	68.5	3	1
C	5 injections, daily	5	1	55.6		

It is quite obvious that the result of the spleen or liver puncture culture cannot be taken as evidence of cure as only 3 out of 29 patients in whose spleen or liver parasites were still present at the end of a fortnight after the last injection had a clinical relapse, and vice versa five whose culture was negative relapsed. But that there is some association between positive cultures and relapses is shown by the fact that the relapse-rate amongst the patients who gave a negative culture was 4.5 per cent, and amongst those giving a positive culture 10.3 per cent.

The leucocyte count — The average count in 53 cases in which various doses, mostly 10 or more, were given on alternate days was 7,545 per c mm. For the cases in which 8 injections were given on alternate days the mean was 7,888 per c mm for the cured patients and 7,250 for the patients subsequently relapsing. In these two groups the counts were done some 10 to 14 days after the completion of treatment. When the concentrated course was introduced the principle of doing a blood count at the end of one week and repeating it at the end of the second week, just before the patient was discharged, was adopted. The first and the second blood counts of the patients who had the concentrated course of 8 daily injections can be grouped as follows —

TABLE IX

The first counts

	Cured	Relapsed
Below 4,000 per c mm	3	
„ 5,000 „ „	5 (or 9.26 per cent)	3 (or 60 per cent)
„ 6,000 „ „	15 (or 27.78 „)	5 (or 100 „)
„ 7,000 „ „	30	
„ 8,000 „ „	38	
„ 9,000 „ „	47	
„ 10,000 „ „	49	
above 10,000 „ „	5	
Mean count of the series	7,003 per c mm	4,997 per c mm

TABLE IX—*concl'd**The second counts*

	Cured	Relapsed
Below 6,000 per c mm	4	0
" 7,000 " "	16	2
" 8,000 " "	28	2
" 9,000 " "	34	4
" 10,000 " "	38	4
above 10,000 " "	11	1
Mean count of the series	8,348 per c mm	8,271 per c mm

A leucocyte count was not done as a routine measure at the time of admission, but from previous experience we know that the average count is below 4,000 per c mm, and in each case in this series in which the count was done it was below this figure. It is thus apparent that in all cases there was a marked increase in the count.

Of the 45 cured cases in which two blood counts were done after treatment, in 35 the second blood count was higher than the first and in 10 it was lower, in each of the 5 relapsing cases in which two counts were done the second blood count was higher than the first. Thus, no special significance can be attached to a rising white-blood-cell count.

Although it is apparent that in the leucocyte count we have no definite criterion of cure, nevertheless the analysis of the first post-treatment counts indicates that there is some association between the leucocyte count and cure. In the relapsing series all the counts were under 6,000 per c mm whereas in the cured series only 27.78 per cent were below this figure. We cannot say that when the first blood count is below 6,000 per c mm the prognosis is bad, as out of the 20 cases in which count was below this figure only 5 (or 25 per cent) relapsed, but we can say that when it is above this figure the prognosis is decidedly good. In the case of the second counts the distinction between the cure and relapsing cases is not so marked and in one relapsing case a count above 10,000 was reported, it seems possible that this patient may have had some inflammatory focus in his body causing this unusual rise in the count.

The fall of temperature—In Group A of the cured cases two were afebrile and five febrile throughout, and the average duration of fever of the 54 others was 8.07

days. Of the relapsing cases one was afebrile and in the other three the average duration of fever was 8 days. In Group B of the cured cases two were afebrile and three febrile throughout and the average duration of fever in the 60 others was 8.02 days, but in this group in the 5 relapsing cases the duration was definitely longer being an average of 12 days for 4 cases cured with the fifth case febrile throughout.

It is thus obvious that, although the duration of the fever is not an absolutely reliable criterion of cure, it is a point that gives some information, as in every relapsing case but one which was not afebrile throughout the fever lasted for more than the average 8 days of the cured cases. Out of 53 cases in which the fever lasted longer than 8 days, 8 relapsed, and in 93 cases in which the fever lasted 8 days or less, one only relapsed.

Splenic enlargement—Taking the cases of Groups A, B and C together there are 46 in which the spleen was not palpable at the conclusion of treatment, of these 4 relapsed. There are 73 in which the spleen was definitely palpable below the costal margin, of these 5 relapsed. And there were 15 in which it was protruding more than an inch below the costal margin, of these one relapsed. Thus, in this series, the size of the spleen would appear to be no criterion of cure whatsoever.

Increase in weight—Again taking the cases of the three groups together, 125 gained in weight, 7 remained the same weight and 9 lost weight. Of the 125 who gained weight, 8 relapsed, and of the 9 who lost weight 2 relapsed. Although the proportion of relapses is greater amongst the patients who lost weight, yet the difference is so slight that this factor gives practically no indication of whether or not a cure is likely to follow.

Conclusions—To summarize one must conclude that there are no immediate criteria of cure, and that the prognosis in any case can be made better by knowing the amount of antimony preparation that has been given than by the employment of any laboratory tests, or by consideration of the clinical progress under treatment.

After an efficient course of treatment the interval in most cases before a 'negative' spleen or liver puncture culture can be obtained is so long that this is not a practical method of prognosis, the interval of 14 days which we have allowed in this series is obviously too short.

No hard and fast rule can be laid down for the leucocyte count, but amongst the cases in which this was done one week after the conclusion of treatment no case with a count of over 6,000 per c mm relapsed, though only 25 per cent of the cases with a count below this figure relapsed.

From the duration of the febrile period a little information can be obtained, in almost every case in which a relapse occurred the fever lasted for a longer period than eight days, but in only about 15 per cent of the cases in which it continued for longer than 8 days did a relapse occur.

From the size of the spleen and the weight of the patient at the time of discharge practically no information of any prognostic value was obtained

OTHER FACTORS INFLUENCING PROGNOSIS

Effect of duration of the disease on prognosis—It is repeatedly stated, even by writers with considerable experience in kala-azar but more frequently by those with little or no practical experience, that if the disease can be treated in its earliest stages a cure can be effected more easily than at a later date. The present writer has for a long time maintained that there is no justification for this assumption which, if based on clinical experience at all is probably based on experience of the treatment in provisionally-diagnosed* cases. In this series, of the patients who had not been treated previously 10 relapsed, of these 9 gave a history of fever for 6 months or less, and 1 gave a history of 1 year's fever. Of one hundred unselected cured cases in the same series 45 gave a history of fever for 6 months or less, whereas 55 gave a history of fever for a larger period than this. This suggests very strongly that early cases do not respond best to treatment, but that the reverse is actually the case. This remark can, however, only apply to previously untreated patients.

The aldehyde reaction before treatment and prognosis—The aldehyde reaction before treatment of a hundred unselected cured cases and the 10 previously untreated relapsing cases were as follows—

Aldehyde reaction	100 cured cases	Relapsing cases
+++	63	3
++	6	
+	9	3
(+) or ±	21	4
—	1	1

That is to say, although 69 per cent of the cured cases originally gave a +++ or ++ reaction, only 3 out of 10 of the relapsing cases gave these reactions

* It is a common practice in one of the large Calcutta hospitals, where admittedly the clinician is handicapped by laboratory facilities which are far short of commensurate with the importance of the institution, to give injections of one of the pentavalent compounds of antimony in all cases of continued fever in which a diagnosis is not made. If they respond to this treatment they are labelled as kala azar.

This procedure which is also adopted extensively in private practice and in other hospitals is not so unsatisfactory for the patients concerned—because these drugs probably raise the resistance generally and are in any case usually harmless—as it is disastrous to the collection of accurate scientific data of the incidence of the disease.

Lloyd, Napier and Paul (1929) have suggested that the aldehyde reaction is evidence of an immunity response. It may, however, simply be additional evidence that early cases do not respond so well to treatment as fully developed ones. The suggestions are not mutually exclusive.

Conclusions —There is no evidence that kala-azar is more amenable to treatment in its early than in its later stages. There is on the other hand some evidence that the reverse is the case.

It seems possible that the degree of reaction occurring in the 'aldehyde test' is an indication of the degree of immunity response occurring in the patient.

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BACTERIOPHAGE IN THE TREATMENT OF PLAGUE

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[Received for publication, July 11, 1931]

INTRODUCTION

VILLAZON (1923) was apparently the first to study the therapeutic property of bacteriophage in experimental animals. While cultivating plague bacilli from living infected wild rats, he found that in two cases, the culture filtrates produced lytic 'plaques' in agar cultures of *B. pestis*. He inoculated two rats, one with bacteriophage and plague bacilli and the other with bacilli only. Both rats died in three days, but on post-mortem examination, the one that had received the phage in addition showed fewer lesions and a smaller number of organisms. He (1923a) then repeated the experiment on three white rats and three guinea-pigs. Out of the three rats, two received both the 'phage and plague bacilli, while the third received only the bacilli. All the three rats died, but on autopsy, there was found a complete absence of plague bacilli in the two which had received both the 'phage and the bacilli. Of the three guinea-pigs again, two were inoculated with bacteriophage and plague bacilli, and the third received only the plague bacilli. One of the two guinea-pigs treated with the 'phage and bacilli survived while the other died and showed only 'one single bacillus' on microscopic examination. In the body of the control animal numerous plague bacilli were found. From these results he assumed that the plague bacilli had been destroyed by the lytic action of the bacteriophage resulting in the release of their endotoxins which, however, proved fatal to all except one guinea-pig, and he suggested the possibility of utilization of bacteriophage in the treatment of plague.

Doorenbos (1926) carried out experiments in rats of the norvegicus species with a strain of plague bacteriophage obtained from d'Herelle. This 'phage was sufficiently virulent to dissolve in 20 to 30 hours a 24-hour broth culture of *B. pestis*. Eight rats were inoculated with 0.1 c.c. of an emulsion of liver from a rat which had died of plague. Four of these rats were fed on bread only, they died after 3 to 4 days and their organs showed typical signs of plague. The other four were fed on bread soaked in 1 c.c. of the 'phage, one of this group survived, while the rest died after 4 to 7 days, but examination of the organs showed that the infection in these was of a less acute character and in one case even the cultures made from the organs remained sterile. In another experiment, 0.2 c.c. of a freshly prepared bacteriophage was injected subcutaneously into four rats which had received at the same time 0.1 c.c. of a broth culture of *B. pestis*. Four others received a dose of 0.01 to 0.1 c.c. of this culture only. The former survived while the latter died after 3 to 4 days.

Compton (1928) carried out experiments with a strain of bacteriophage also obtained from d'Herelle. It was not one of 'maximum virulence' which, as defined by d'Herelle, is able to lyse completely in 4 to 5 hours at 37°C a 1 to 2 thousand million suspension of *B. pestis* in broth. It, nevertheless, possessed a virulence such as a few drops added to a broth tube lightly seeded with *B. pestis* completely inhibited the growth of the latter. Further, when added to a 24-hour broth culture of *B. pestis* diluted 1 in 2 with fresh broth just before the addition of the 'phage, it led to clarification of the culture in 24 to 36 hours. A sufficient stock of this 'phage was then prepared. Six adult mice of average weight of 30 grammes were infected subcutaneously with 0.1 to 0.2 c.c. of a 24-hour broth culture of *B. pestis* diluted 1 in 2 immediately before use. Each animal then in turn received respectively 1, 2, 3, 24, 48 and 48 hours later, 0.5 to 1 c.c. of anti-plague bacteriophage in the rump. All the animals succumbed in 2 to 3 days with typical appearances of experimental plague. He, therefore, concluded, that subcutaneous injection of the specific 'phage was without any curative effect in experimental infection.

Flu (1929) instituted therapeutic tests in guinea-pigs which received one hundredth of a loopful of a 24-hour plague culture subcutaneously, the treatment consisted of the administration of 1 c.c. of the 'phage subcutaneously, immediately after infection and after an interval of 24 and 48 hours. His results were entirely negative. Oral administration and subcutaneous injection of bacteriophage in white rats also gave little evidence of curative value.

D'Herelle (1925) reported the successful treatment of four cases of bubonic plague in Alexandria with a bacteriophage originally isolated by him from the droppings of a rat in Indo-China. This was brought to the notice of the Government of India. A sufficient supply of this 'phage was obtained from him in March 1926, to carry out therapeutic tests on human cases of plague in India. Between the 16th March and the 30th April, 1926, we (Naidu and Avari, 1927) carried out

treatment with this 'anti-pestiphage' on 103 cases with 97 controls. The diagnosis of plague was established by cultural methods. From the results, we concluded that (1) septicaemic cases of moderate or severe degree did not recover whether treated with the bacteriophage or not, and (2) treatment with this 'phage had no influence either on the course of the disease or on the case mortality even in cases which were mildly septicaemic or purely bubonic in character. While the above experiments were in progress, d'Herelle came over to Bombay and as soon as the unsatisfactory nature of his bacteriophage became evident, he proceeded to search for a more virulent strain of the 'phage from the droppings of rats trapped in this city. He isolated a bacteriophage which lysed a 24-hour broth culture of *B. pestis* in about eight hours. With this bacteriophage experiments involving the use of 40 Madras rats, which are highly susceptible to experimental infection with plague, were carried out. About 3 c.c. of this bacteriophage was fed to each of the 10 Madras rats which had no food or water for 24 hours. All drank it readily. This was followed by the administration of 0.003 mg. of the spleen of a rat that had died of acute plague. All the 10 rats died of plague, 7 on the third and 3 on the fourth day after infection. Three batches of 10 Madras rats each, received 0.003 mg. of plague spleen subcutaneously. The first batch received 0.1 c.c. and the second batch 0.04 c.c. of the bacteriophage immediately after infection, the third batch received no bacteriophage. There were no survivors from any of the three batches by the 8th day of infection. Thus, even this 'phage which caused lysis of a broth culture in about 8 hours had no therapeutic effect. From these results, d'Herelle concluded that the bacteriophage sent out from Alexandria and the one locally isolated by him were not sufficiently virulent to deal with the strains of *B. pestis* in India which seemed to him to be 'extraordinarily virulent'. He, therefore, recommended that 'researches be made in India with the object of obtaining a bacteriophage able to produce complete lysis of a 24-hour virulent culture of *B. pestis* (local strains) at a temperature of 38°C, within the shortest time of contact, viz., 4 to 6 hours. As bacteriophage virulent for *B. pestis* is found in the intestinal contents of rats which resist infection with plague and also in the intestine and in the buboes of men during recovery from plague, a virulent bacteriophage should be isolated from these sources, but of the two a human source seems preferable. It would be necessary for this study to examine the contents of the bubo and intestinal contents in man daily from the commencement of disease to the period of recovery, as the presence of bacteriophage is often transient'.

One of us (C. R. A.) had the opportunity of learning the technique of isolating the 'phage from d'Herelle while he was in Bombay. He, therefore, carried out a series of experiments extending over several months to isolate an active bacteriophage from rats and from human cases of plague admitted to the Maratha Plague Hospital, Bombay. The 'phage was looked for in the

buboes, internal organs and droppings of rats which had survived an experimental infection with plague and in the discharges from buboes and in the faeces of convalescing plague patients. Bacteriophage of varying virulence was found ten times in the 1,242 examinations made. Finally a 'phage was isolated from buboes and spleen of a rat which lysed a broth culture of *B. pestis* of 24 hours' growth in less than two hours. This lysed culture was filtered through a Chamberland filter candle (L_3) and a few drops of this filtrate were added to another 24 hours' broth culture. Lysis occurred in this also in less than 2 hours. This 'phage was now maintained by successive passages in suspensions of plague bacilli in broth containing one to two thousand million organisms.

BACTERIOPHAGE THERAPY OF PLAGUE IN EXPERIMENTAL ANIMALS

Having secured an active 'phage against our local strains of virulent plague bacilli, one of us (B. P. B. N.) proceeded to study its therapeutic value in experimentally infected laboratory animals.

Experimental animals—Of the laboratory animals rats, guinea-pigs and rabbits are susceptible to an experimental infection with plague. In these animals we have uniformly obtained a mortality of 80 to 100 per cent following the subcutaneous injection of our test dose, namely, 0.003 mg. of spleen of a rat that had died of acute plague. It is generally held that guinea-pigs are not suitable animals for carrying out experiments on the prophylaxis and treatment of plague and our own observations confirm this view. As regards the rat, we have observed in our experiments on the therapeutic value of different anti-plague sera, that in the highly susceptible Madras rats deaths continue to occur among them irregularly over a prolonged period even after all the controls are dead until practically no survivors are left thus making it very difficult at the end of the period of observation to gauge the relative value of the particular serum employed. In the case of the rabbit, however, the results obtained have been fairly consistent. When once the infected control animals were dead, no further deaths among the treated animals occurred even though the period of observation was extended to 30 days or longer. The rabbit, therefore, was found to be the most suitable animal for testing the therapeutic value of anti-plague sera. It has this further advantage, namely, that it readily offers itself to intravenous medication, a method which seems necessary in a septicæmic disease like plague. In the case of brown rats this mode of administration could be employed only with difficulty. For these reasons, we chose rabbits as our test animals in these experiments.

Test dose—From Table I it will be seen that in rabbits the mortality following the subcutaneous injection of our usual test dose varied between 80 and 100 per cent with an average of 92 per cent. It was suggested that this test dose was too severe, in as much as the average mortality in human epidemics is usually only about 70 per cent as compared with the mortality of 92 per cent in experimentally

TABLE I

Mortality following on the subcutaneous injection of 0.003 mg of spleen of a rat which has died of acute plague (Dose of our test virus)

Year	Number of rabbits	Daily mortality following on infection															Total deaths in 30 days after infection	Percentage mortality
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1926	16	0	0	5	0	5	3	1	0	0	0	0	0	0	0	0	14	87.5
1927	17	0	0	3	5	2	1	2	1	1	0	0	0	0	0	0	17	100.0
1928	26	0	0	7	6	7	2	1	0	0	0	0	1	0	0	0	24	92.3
1929	41	0	2	25	6	1	3	1	2	1	0	0	0	0	0	0	41	100.0
1930	45	0	0	4	11	8	6	5	2	1	0	1	0	0	0	0	38	84.4
TOTAL	145	0	2	16	28	23	15	10	5	3	0	1	1	0	0	0	134	92.4

infected rabbits From our previous experience we knew that with diminishing doses of our test virus the interval between infection and the death of the animal becomes prolonged and that some of the infected animals even escape death Yet in response to this suggestion we employed in these experiments very much smaller test doses, namely, one thousandth or one ten-thousandth of our usual dose

Administration of the 'phage—We have mostly employed the 'phage intravenously at varying intervals after infection

Diagnosis of the cause of death—The animals that died after infection were carefully examined As we have observed on several occasions that even though on microscopical examination no plague bacilli are detected in smears yet when cutaneous infection in rats is made with the spleen of the dead animal it results with very few exceptions in the death of the passage animal with all signs of acute plague We have, therefore, made it a rule always to confirm the post-mortem findings by animal passage

Results of experiments—The experiments are summarized in Tables II, III and IV

The results of these experiments show that (1) with one thousandth part of our usual test dose, the mortality among the treated and the control rabbits has been the same whether the treatment was commenced immediately after infection or was delayed by 24 or 48 hours, (2) with one ten-thousandth part of our test dose, there was a reduction in the mortality among the animals treated 72 hours after infection by 10 per cent But this difference can easily be accounted for by the smallness of the infecting dose which results in the escape of some animals as already mentioned, (3) with the reduction in the amount of the test dose employed the average mortality among the controls fell from 92.4 to 69.0 per cent, (4) neither the quantity of the 'phage injected nor its repetition had any influence on the course of the disease or on the case mortality

While these experiments were in progress, a supply of an anti-plague serum prepared from rabbits became available We, therefore, carried out an experiment to test if the combined administration of the 'phage along with the anti-plague serum had any advantage over the use of the serum alone The results of this experiment are summarized in Table V From these, it would appear that the administration of the 'phage along with the serum had not only no advantage but on the contrary had the effect of lowering the curative value of the serum in as much as the mortality following the administration of the serum alone was only 25 per cent, while the combined use of the same amount of serum with the bacteriophage increased the mortality to 50 per cent, the mortality among the control animals being 100 per cent

With the kind co-operation of D. P. T. Patel, the Superintendent of the Maratha Plague Hospital, Bombay, one of us (C. R. A.) carried out the treatment of plague in 33 patients with this 'phage

TABLE II

Bacteriophage therapy in rabbits experimentally infected with plague

Treatment was commenced either immediately after infection or after an interval of 24 hours, test dose employed was one thousandth part of the usual dose, namely, 0.003 mg of spleen of a rat died of acute plague, doses of bacteriophage administered are in cc

Weights of rabbits in grammes		DAILY MORTALITY AMONG TREATED RABBITS										Deaths within 30 days after infection	DAILY MORTALITY AMONG CONTROL RABBITS										Deaths within 30 days after infection																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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Note—S = Injected subcutaneously

I V = Injected intravenously

D = Died and when the spleen was passed into a rat, it also died of plague

d = Died, but the passage rat survived for 15 days from the date of infection

Mortality among rabbits treated immediately = 72.2 per cent

Mortality among rabbits treated 24 hours after = 65.6 per cent

Mortality among control rabbits = 71.4 per cent

TABLE III

Bacteriophage therapy in rabbits experimentally infected with plague

Treatment was commenced 48 hours after infection, bacteriophage was administered intravenously, test dose employed was one thousandth part of the usual dose which is 0.003 mg of spleen of a rat died of acute plague

DAILY MORTALITY AMONG TREATED RABBITS												Deaths within 30 days after infection
0	1	2	3	4	5	6	7	8	9	10	11	
Weights of rabbits in grammes												Deaths within 30 days after infection
1,520												1
1,520	0.5											1
1,520	0.5											1
1,300	1											1
1,300	1											
1,870	1											
1,500	2											
1,520	2											
1,530	2											1
1,530	2											1
1,870	3											1
1,470	3											1
1,850	4											1
1,570	4											
1,400	1											
1,400	1											
1,800	1											
1,450	2											1
1,570	2											1
1,770	3											1
1,520	3											1
1,750	4											1
1,450	4											1
1,200	1											
1,300	1											
1,750	1											
1,750	2											
1,570	3											
1,750	3											
1,570	4											
1,570	4											
30	0	0	0	0	1	3	3	6	2	1	2	1
												20

DAILY MORTALITY AMONG CONTROL RABBITS												Deaths within 30 days after infection
0	1	2	3	4	5	6	7	8	9	10	11	
Weights of rabbits in grammes												Deaths within 30 days after infection
1,900												1
1,600												1
1,700												1
1,700												1
1,800												
1,700												
2,000												
1,970												1
1,700												1
1,470												1
1,450												1
1,420												1
1,700												
1,800												
1,670												
1,650												
1,620												
18	0	0	0	1	2	2	1	1	0	2	1	12

See note under Table II
Doses of bacteriophage administered are in c.c.
Mortality among treated rabbits = 66.6 per cent
" " control " = 66.6

D. B. Horsberg, J. Horsberg in rabbits experimentally infected with plague

Bacteriophage therapy in the rat

Treatment was commenced 72 hours after infection, bacteriophage was administered intravenously, test dose employed was one ten-thousandth part of the usual dose which is 0.003 mg of spleen of a rat died of acute plague

[illegible]

TABLE

*Comparative results following the combined use of anti-pestiphage and
of rabbits experimentally*

*Treatment was commenced soon after and at varying intervals
which died of*

Weights of rabbits in grammes	DAILY MORTALITY AMONG TREATED RABBITS														Deaths within 30 days after infection	Weights of rabbits in grammes	DAILY MORTALITY							
	0			1			2		3		4		5	6			7		0		1		2	
	'Phage S C	Serum S C	Serum I V	'Phage S C	Serum S C	Serum I V	'Phage S C	Serum I V	'Phage S C	Serum I V	'Phage S C	Serum I V	Serum I V	Serum I V			Serum S C	Serum I V	Serum I V	Serum S C	Serum I V	Serum S C	Serum I V	Serum S C
1,970	1	5	1	1	5	1	1	2		2		2	2	2	5		2,050	1	5	1	5	2		
1,670	1	5	1	1	5	1	1	2		2		2	2	2	5		1,750	1	5	1	5	2		
1,950				1		2	1	2	1	2		2	2	2		2	2,000			1	5	1	5	
1,770				1		2	1	2	1	2		2	2	2		2	1,700			1	5	1	5	
2,010							1	2	1	2	2	4	d				1	1,995					1	5
1,780							1	2	1	2		d					1	1,700					1	5
2,050									1	2	1	2	d				1	2,000						
1,820									1	2	1	2	d				1	1,630						
8	0			0			0		0		1		3	0	0		4	8	0		0		0	

See note under Table II

Doses of 'phage or serum administered are in c.c.

Mortality among rabbits

" " "

" " untreated

V.

immunized rabbit serum and of immunized rabbit serum only in the treatment
infected with plague

of infection, the test dose employed was 0.003 mg of spleen of a rat
acute plague

AMONG TREATED RABBITS										Deaths within 30 days after infection	Weights of rabbits in grammes	DAILY MORTALITY AMONG CONTROL RABBITS													Deaths within 30 days after infection	
3		4		5		6	7		8			9	1	2	3	4	5	6	7	8	9	10	11	12		13
Serum I V	Serum S C	Serum I V	Serum S C	Serum I V	Serum S C	Serum I V	Serum I V	Serum S C																		
2		2		2		2		5			2,080						D								1	
2		2		2		2		5			1,830				D										1	
2	5	2		2		2	2				2,080		D												1	
2	5	2		2		2	2				2,075												D		1	
1	5	1	5	2		2	2		d	1	1,630				D										1	
1	5	1	5	2		2	2				1,570					D									1	
1	5	1	5	1	5	d				1	1,570					D									1	
1	5	1	5	1	5	2	2				2,000		D												1	
0		0		0		1	0	0	1	2	8	0	0	2	1	1	2	1	0	0	0	0	0	1	8	

treated with phage and serum — 500

treated with 'phage and serum = 50.0 per cent

" " serum only = 25.0 "

control rabbits = 100.0 "

The results have been reported by him (Patel, 1929) as follows —

‘Each patient received *Bacteriophage pestis* both intravenously and in the bubo twice a day for two days after admission. The results were unsatisfactory, all the patients died’

‘Details of the treatment by *Bacteriophage pestis* —

- | | | |
|-----|--|-------------------------|
| I | 10 c c intravenously and 5 c c in the bubo to 6 patients | 4 received 4 injections |
| | | 1 „ 2 „ |
| | | 1 „ 1 injection |
| II | 5 c c intravenously and 2 c c in the bubo to 7 patients | 1 received 4 injections |
| | | 2 „ 2 „ |
| | | 4 „ 1 injection |
| III | 2 c c intravenously and 1 c c in the bubo to 14 patients | 7 received 4 injections |
| | | 1 „ 2 „ |
| | | 6 „ 1 injection |
| IV | 2 c c by mouth and 1 c c in the bubo to 6 patients’ | |

SUMMARY

1 The anti-plague bacteriophage having a rapid lytic action upon cultures of local virulent strains of plague bacilli *in vitro* was found to be of little value when used in the treatment of plague either in experimentally infected animals or in cases of natural infection in man

2 When used in combination with an anti-plague serum in the hope that its strong lytic action upon plague bacilli will enhance the curative effect of the serum it was found that the combined use resulted in a lesser number of recoveries from plague than what followed the use of the serum alone

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‘GERMANIN’ (‘BAYER 205’) IN THE TREATMENT OF PLAGUE.

BY

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[Received for publication August 15 1931]

INTRODUCTION

EVER since the outbreak of bubonic plague in India almost every drug which had been favourably reported upon as a possible cure has been tried at the Maratha Plague Hospital, Bombay, but with disappointing results. Choksy (1923) after an experience extending over a period of more than 25 years in the treatment of plague came to the conclusion that ‘all efforts hitherto made to cure the disease by drugs have proved absolutely futile’ and urged the necessity for undertaking further researches into the possibility of preparing a potent anti-plague serum in India, as ‘it is the only remedy that holds out any hope of reducing the excessively high case mortality which has so markedly characterized the epidemics at Bombay. Patel, the present Superintendent of the Maratha Plague Hospital also came to the same conclusion. He (Patel, 1925) observes that chemotherapy of plague with iodine and its preparations, carbolic acid, mercurochrome, salvarsan preparations, etc., has not been found to have any effect either on the mortality or on the course of the disease. Looking to the analogy of other infectious diseases, the only rational treatment, in his opinion, would be with a powerful concentrated antitoxic serum prepared from local strains and given early and in large doses. Until such a potent antitoxic serum becomes available, the medical practitioner called upon to treat cases of plague must necessarily have recourse to one or the other of the drugs which have been commended by his fellow practitioners.

Dr O Urchs (1930) of Messrs Bayer-Meister Lucius, having received a communication from Dr Vincent of Maymyo of the very good results he had obtained with '*Bayer 205*' in the treatment of some cases of plague, suggested that he would place a sufficient supply of the drug at our disposal should we desire to test its therapeutic value in experimental animals. This paper deals with a brief history of chemotherapy of plague and with our experiments with '*Germanin*,' a preparation of '*Bayer 205*' for human use, in rabbits—animals susceptible to experimental infection with plague.

CHEMOTHERAPY OF PLAGUE

Several drugs have been administered to plague patients either orally, subcutaneously or intravenously, not only to relieve the distressing symptoms which accompany the disease but also with a view to destroying the plague bacilli in the body and effecting a cure. With this latter object, Choksy introduced the method of injecting germicides into buboes during the first epidemic of plague in Bombay.

Carbolic acid—Choksy (1904) injected either pure carbolic acid or equal parts of carbolic acid and liquor iodi in doses of 10 to 20 minims into each bubo. Paton (1908) reported successful results following oral and subcutaneous use of carbolic acid among cases treated at Amoy. Choksy (1918) found that even when pure carbolic acid was administered in doses of 72 to 100 grains per day, it was inert. Patel (1925) also obtained no success with this drug.

Formalin and its preparations—Rochr (1912) suggested the injection of a two per cent solution of formalin into the bubo and Deggeller (1915) treated 5 cases of plague with intravenous injections of formaldehyde sodium bisulphite ('*Fonabist*') with 3 deaths, but the number of cases treated was too small to allow of any definite conclusion with regard to its therapeutic value.

Eusol—Connor (1916) suggested the subcutaneous injection of eusol in the treatment of plague. Brayne (1917) and Baker (1920) employed eusol but with no benefit.

Izal—Choksy (1904) treated 21 cases of plague with injections of 5 to 10 c.c. of a one per cent solution of izal into buboes, he found that these injections had no influence, either local or general, on the course of the disease or on the mortality. Patel (1926) also employed izal without any benefit.

Mercury and its preparations—Choksy (1918) treated 21 cases of plague by subcutaneous injections of *collosol mercury* with 15 deaths. Andrew Balfour (1924) suggested the use of *mercurochrome-220 soluble* in plague. He (1925) reported later that he had received accounts of a few cases of plague in which the drug had been distinctly beneficial. Patel (1925) treated 6 cases of plague with this drug by the intravenous route and all ended fatally. Still later, he (1929) tried the drug on two more cases but without benefit. We (Naidu and Shamsher Jung, 1926) found that in

experimentally infected animals, such as rats and rabbits, the injections of *mercurochrome-220* soluble in sublethal doses either single or repeated had no influence on the case mortality. At the Haffkine Institute Calcutta, Kamath and Naidu (1927) tested the bactericidal action of some organic compounds of mercury on '*B. pestis*' *in vitro* and found that the bactericidal power of these drugs was roughly proportional to the amount of mercury present in each. One of us (B P B N) tested the therapeutic action of *tetra-acetoxy-mercuri-trypan blue*, a synthetic preparation prepared at this Institute, in experimentally plague infected rabbits as it exhibited very high germicidal property against '*B. pestis*' *in vitro*. But when tested *in vivo* the drug was found to have no influence on the mortality from the disease in these animals.

Silver and its preparations —Elliot (1907) reported successful cures in bubonic plague following the intravenous injections of a one per cent solution of collargol. Choksy (1918) employed *colloidal silver* in 93 cases of plague with 60 deaths (64.5 per cent). Denman (1914) found that early cases of plague were benefited by intravenous injections of *electrargol*. In his series the mortality among the treated cases was 60.4 per cent as compared to the mortality of 83.4 per cent among controls. Ilvento and Mazitelli (1914) and Choksy (1918) also employed *electrargol* in the treatment of plague but found the drug to be of little value.

Salvarsan and allied arsenical preparations —Lancelotti (1912) and Aumann (1912) employed intravenous injections of salvarsan without benefit in plague. Schut (1921) treated 5 cases of plague by intravenous injections of *neosalvarsan* all of which ended fatally. Ram Mansoor (1922) treated one case of plague with the same drug and it recovered. Marshall and Achhru Ram (1922) reported successful cures in 6 out of 7 cases treated with *neokharsvan*. Patel (1925) found that salvarsan preparations had no effect on the case mortality of plague.

Iodine and its preparations —Choksy was apparently the first to use iodine in the treatment of plague. He (Choksy, 1901) treated 104 cases by the oral administration of *iodine terchloride* with a mortality of 66.3 per cent. Patel (1925) also treated 32 cases with the same drug administered orally and had a mortality of 87.5 per cent. Commissioner Booth Tucker of the Salvation Army (1913) reported successful cures following oral administration of *tincture of iodine*. Connor (1912-13) advocated the use of this drug intravenously in plague and reported that all the three cases he had treated recovered. Liston (1913) conducted an experiment with tincture of iodine among cases admitted to the Maratha Plague Hospital. He classified the cases into four groups according to bacteriological findings: (1) no plague bacilli in 0.25 c.c. of blood, (2) some but less than 10 plague bacilli in 0.25 c.c. of blood, (3) more than 10 but less than 100 bacilli in 0.25 c.c. of blood, and (4) more than 100 bacilli in 0.25 c.c. of blood. He considered such a classification necessary as he had previously observed that all patients who showed more than 10 plague bacilli in 0.25 c.c. of blood invariably died, while a proportion of cases with no plague

bacilli or less than 10 bacilli in 0.25 c.c. of blood recovered. In his series of 60 cases, 20 received the routine treatment adopted by Choksy in his hospital cases, 20 were treated with tincture of iodine by the mouth while 20 were given the drug intravenously. In the first group of 20 cases there was a mortality of 16, in the second 12 and in the last group 17. Among the cases in which recovery was considered possible from bacteriological findings as explained above, in the first group there were 9 cases with 55.5 per cent mortality, in the second there were 12 cases with 33.3 per cent mortality and in the last there were 10 cases with 70 per cent mortality. As the number of cases treated in each group was not sufficiently large, he could not come to any definite conclusion as to the value of this treatment. Choksy (1914) in view of the lesser mortality that followed oral administration of tincture of iodine among the cases treated by Luston, treated 27 cases by the same method and had a mortality of 81.5 per cent. He (Choksy, 1915) made a further trial with tincture of iodine in 88 cases and had a mortality of 72.7 per cent. From these results he concluded that 'plague as observed in Bombay is unaffected and not amenable to treatment by iodine'. Rama Iyer (1916) treated 6 cases of plague with intravenous injections of tincture of iodine and of these 5 recovered. He also reported that Mg Tha Din treated a few cases in Mandalay by the same method with satisfactory results. Vassallo (1921) treated 20 cases by intravenous injections of tincture of iodine with 4 deaths and attributed his good results to very early treatment. Jendwine (1923) found that early cases were benefited by intravenous injections of tincture of iodine and stated that Hari Ram had reported 26 cures out of 28 cases treated with intravenous injections and that Gian Chand also reported in the same manner. Pal (1924) treated 2 cases with intravenous injections of iodine, of these one died. He noticed that the injections of iodine resulted in the reduction of the temperature of plague patients even in septicæmic cases but 'as regards cures the results are as disappointing as with any other form of drug treatment of this dreaded disease'. Naquvie (1924) reported that he had treated about 100 cases of plague by intravenous injections of iodine and that most of his cases recovered and estimated the cures at 75 per cent. Patel (1922) employed 0.6 per cent solution of iodine subcutaneously and also intravenously in 153 cases with a mortality of 73.2 per cent, while the mortality among the 48 control cases was 72.7 per cent. He (Patel, 1924) further reported that he had treated in all 380 cases with tincture of iodine by oral, subcutaneous and intravenous routes with a mortality of 75.7 per cent. Marshall and Ram (1924) reported that the latter had treated 103 cases of plague in Uganda by intravenous injections of iodine and only 16 of these recovered, and that Baker had treated 50 cases by the same method and of these 19 recovered. Bhaikadway (1926) reported that he had treated about 100 cases by the same method and claimed 80 per cent cures. Mallanah (1920) advocated the injection of a solution of iodine, camphor and thymol into the plague bubo and reported that out of 34 cases treated by this method in the Hyderabad Isolation Hospital there were 15 deaths whereas

when tincture of iodine was injected into the bubo and also orally, all the twenty cases thus treated died. Subbiah Pillai (1921) treated 3 cases by Mallanah's method with 2 deaths. Carman (1927) reported that he had treated 27 cases by the same method with 9 deaths (33.3 per cent). Patel (1929) treated 16 cases by intravenous injections of this mixture with 14 deaths (87.5 per cent). Choksy (1920) reported that he obtained good results following the use of colloidal preparations of iodine. Out of 260 patients treated with these preparations there were 160 deaths (61.5 per cent). Grimes (1926) treated seven cases of plague with *colloidal iodine* and of these only one died. Choksy (1918) treated 63 cases with *iodol* and had 41 deaths (65.1 per cent).

From this review, it is apparent that a very large number of cases of plague have been treated especially with iodine in one form or another but with very divergent results—some claiming cures with this drug to the extent of 80 to 100 per cent while in the hands of others the results have been very disappointing. In spite of these apparently conflicting results iodine continues to be a favourite remedy with many. This is partly due to the fact that its use is unattended with any constitutional disturbance and that a temporary fall in the temperature of the patient very often follows its injection. The divergent results obtained by these several observers may be reconciled when one remembers that in the course of an epidemic several cases of fever resembling plague but in reality not due to infection by *B. pestis* are treated as such. In a series of 276 cases that were sent to the hospital by medical practitioners in which we carried out bacteriological examination both by bubo puncture and by blood culture there were no less than 40 cases in which the diagnosis of plague could not be confirmed. It is obvious that inclusion of such cases among those treated as plague with any special line of treatment will naturally raise the percentage of recoveries depending upon the proportion of such cases treated. Further, even among cases that have been definitely diagnosed as plague and classified according to the severity of blood infection, our observations have shown that in purely bubonic cases in which no plague bacilli were detected in 0.25 c.c. of blood the mortality under ordinary hospital treatment varied only from 25 to 33 per cent, while in septicæmic cases in which less than ten plague bacilli were present in 0.25 c.c. of blood the mortality rose to about 75 per cent and in septicæmic cases in which more than ten bacilli were detected in the same amount of blood the mortality was 100 per cent. From these results it would be evident that for correctly estimating the value of any form of treatment it is necessary not only to confirm the diagnosis of plague by bacteriological examination but also to estimate the severity of infection in each case.

‘GERMANIN’ (‘BAYER 205’)

‘Germanin’ is a loose white powder which dissolves readily in physiological solution of sodium chloride and also in cold distilled water. According to the

manufacturers it is a complex organic combination containing neither arsenic, antimony, mercury nor any other inorganic substance. It is regarded as the best trypanosomicidal drug at present available for the treatment of human trypanosomiasis. The manufacturers emphasize that this drug has produced no satisfactory results in other diseases not caused by trypanosomes. It has been ascertained that in encephalitis lethargica, it has proved to be quite useless, while in other bacterial diseases and in infections caused by spirochaetes it has not been a success. They warn that the drug is not entirely harmless. They, therefore, recommend not to employ it at random, as it would not only do harm but also discredit the drug.

Nevertheless, this drug has been used by some observers for the treatment of plague. Dyce Sharp (1926) reported 8 recoveries out of the 12 cases of plague he had treated with 'Bayer 205,' while Carman (1927) found that this drug had no definite specific action on plague. Very recently, Vincent (1931) published successful results he obtained in four cases of plague with 'Bayer 205'. In view of these apparently good results which are reported to have attended the use of 'Bayer 205' in the treatment of plague we undertook to test this drug in experimental animals.

OUR EXPERIMENTS WITH 'GERMANIN'

(1) Germicidal action of 'Germanin' on *B. pestis*

In the first instance, we attempted to ascertain the germicidal property of this drug on the plague bacillus. A broth culture of *B. pestis* of 48 hours' growth was employed. Equal volumes of this culture and of different dilutions of 'Germanin' were mixed together and allowed to stand at room temperature. At the end of 15 minutes and 24 hours respectively, cultures were made from these mixtures on blood-agar slopes. These subcultures were examined for growth from day to day for a period of seven days. The results showed that the drug has no germicidal action on *B. pestis* even in a dilution of 1 in 20 and after a contact of as long as 24 hours.

(2) Toxicity of 'Germanin' for rabbits

We then proceeded to test the toxicity of the drug. For this purpose rabbits weighing from 1,690 to 1,980 grammes were inoculated intravenously with a single dose varying from 0.01 to 2 grammes of the drug. In another series, rabbits weighing from 1,690 to 2,790 grammes received intravenous injections of the drug in doses varying from 0.1 to 0.5 gramme repeated at intervals of 24 hours for 2 to 7 days, the total amount of the drug injected being 0.7 to 1.25 grammes. The results showed that the drug was not toxic to rabbits up to 0.6 gramme, given either as a single dose or in fractional doses, but was fatal when the dose reached 0.7 gramme and over with a mortality of 100 per cent, death ensuing within about 20 minutes to seven days according to the size and fractioning of the dose employed. These results are summarized in Tables I and II.

From Tables I and II it would also appear that either a single dose of 0.5 gramme or repeated doses of 0.1 gramme on five successive days at intervals of 24 hours were well borne by rabbits.

Following the injection of a rapidly fatal dose rabbits scream, have convulsions, their respirations become hurried and death ensues in a short time. Should the animal survive for a period of 24 hours or longer, as it happened when the doses were given in fractions, the post-mortem appearances in these animals were (a) hæmorrhages in the walls of the intestines, more particularly in the large intestine, (b) liver presenting appearances of simple congestion to fatty degeneration, (c) spleen congested, (d) lungs presenting appearances of congestion or sometimes of broncho-pneumonia, and (e) pleural, pericardial and in one instance peritoneal effusion.

TABLE I

Weight of rabbits in grammes	Daily mortality following intravenous injections of 'Germanin' in single dose												Survivors at the end of 30 days after treatment	
	0	1	2	3	4	5	6	7	8	9	10	11		12
1,730	gm 0 01													1
1,750	0 0125													1
1,690	0 025													1
1,750	0 05													1
1,800	0 05													1
1,820	0 1													1
1,820	0 125													1
1,830	0 25													1
1,900	0 5													1
1,800	0 5													1
1,900	0 6													1
1,910	0 7	Died within one hour after injection												D
1,900	0 8	do	do	20 minutes		do	do							D
1,940	1 0	do	do			do	do	do						D
1,980	2 0	do	do			do	do	do						D
TOTAL 15														11

D = Died

TABLE II

Weight of rabbits in grammes	Daily mortality following intravenous injections of 'Germanin' in repeated doses										Survivors at the end of 30 days after treatment	
	0	1	2	3	4	5	6	7	8	9		10
	gm	gm	gm	gm	gm	gm	gm					
1,820	0.5	0.5	D									
1,800	0.5	0.5	D									
1,750	0.5	0.5	D									
1,690	0.5	0.5	D									
1,870	0.25	0.25	0.25	0.25	D							
1,990	0.25	0.25	0.25	0.25	0.25		D					
2,020	0.25	0.25	0.25	0.25	0.25	D						
2,070	0.25	0.25	0.25	0.25	0.25		D					
2,010	0.1	0.1	0.1	0.1	0.1							1
2,220	0.1	0.1	0.1	0.1	0.1							1
2,220	0.1	0.1	0.1	0.1	0.1							1
2,230	0.1	0.1	0.1	0.1	0.1	0.1						1
2,790*	0.1	0.1	0.1	0.1	0.1	0.1	0.1					D
TOTAL 13												1

* Died seven days after the last injection D = Died

(3) *Treatment of plague in experimentally infected rabbits*

Rabbits were infected subcutaneously with our test dose, namely, 0.003 milligram of spleen of a rat dead of acute plague. They were treated with varying amounts of 'Germanin'. In one series, the first dose was injected immediately after infection. Some of the infected animals received a single dose while in others the doses were repeated at intervals of 24 hours. Out of 10 rabbits treated with a single dose varying from 0.1 to 0.5 gramme intravenously, all died by the eighth day after infection. Of the 10 rabbits treated with repeated doses in total amounts varying from 0.3 to 0.5 gramme, 9 died by the twelfth day of infection, while the mortality among the ten control rabbits was 80 per cent during the same period. In another series, treatment was commenced after 24 hours of infection and the

TABLE III

Treated with 'Germann' intravenously mortality following infection												Percentage of mortality at the end of 30 days after infection	Weight of rabbits in grammes	Treated with anti plague serum intravenously Daily mortality following infection						Percentage of mortality at the end of 30 days after infection	Weight of rabbits in grammes	Untreated controls mortality following infection						Percentage of mortality at the end of 30 days after infection	Weight of rabbits in grammes																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																				
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0	1	2	3	4	5	6	7	8	9	10	11			0	1	2	3	4	5			6	1	2	3	4	5			6	7	8	9																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
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D=death from plague

infected animals received three doses of 0.1 gramme of the drug at intervals of 24 hours. All the five rabbits so treated died by the eighth day after infection.

For purposes of comparison, ten other infected rabbits were treated at the same time with our local anti-plague serum. All these serum-treated animals survived, while four out of the five control rabbits died within 9 days after infection. These results are summarized in Table III.

CONCLUSIONS

1 'Germanin' ('Bayer 205') when tested *in vitro* was found to possess no germicidal action upon *B. pestis* even in a dilution of 1 in 20.

2 The minimum lethal dose for rabbits was found to be 0.7 gramme whether given as a single dose or in fractional doses administered in the course of 2 to 7 days.

3 When tested in experimentally infected rabbits in tolerant doses it was found to possess no curative property against plague.

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IS PARAGONIMIASIS LIKELY TO SPREAD IN INDIA?

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[Received for publication, July 18, 1931]

IN attempting an answer to this interrogative title, it must first be pointed out that India falls under the group of countries described as showing a sporadic distribution of Paragonimiasis (Lane and Low, 1922). The few cases on record are (a) Cobbold (1859) described *P compactus* from *Viverra zibetha*, (b) Leiper (1913) recorded *P westermanni* from India, and suggested that *P compactus* and *P westermanni* were synonymous, the former being the valid name on account of priority, (c) Surveyor (1919) found *Paragonimus* eggs in the sputum of a Chinaman, evidently an imported case, at Bombay, (d) Ververs (1923) redescribed *P compactus* from the Indian mongoose, and also recorded *P westermanni* and *P kellicotti* from *Felis tigris*, and *Felis bengalensis* in the London Zoo, (e) Gulati (1926) discovered a new species *P edwardsi* from a new host, *Paradoxurus grayi*, in the Kumaon hills, Himalayas, (f) in 1926, H Cooper, Pathologist, Imperial Institute of Veterinary Research, Muktesar, collected pieces of lung containing cysts which the writer identified as caused by the flukes of the genus *Paragonimus* from a civet-cat *Viverra zibetha*, a hitherto unrecorded host *

To sum up, the disease exists in India among wild animals, and has been noted in two imported human cases. These stray records suggest that either (1) there is actual sparsity of the disease, or (2) there is lack of investigation. The truth of the two possibilities can be verified by future investigations alone.

Since the validity of species from man as distinct from those described from wild animals has been doubted, and specificity of host relationships are not much adhered to by some helminths infecting man, the question whether the sporadic distribution noted above threatens human health or that of his livestock to any

* This collection was made from Gauhati, Assam, by Mr H Cooper. Other worms collected from the same host were identified by the writer as *Ankylostomum braziliense* and *Rictularia* sp. Dr H A Baylis of the British Museum in a private communication suggested that this *Rictularia* sp was possibly a new species although closely allied to *R. cahirensis jagerskiold*.

extent merits consideration at the hands of the experts and authorities. As far as wild animals are concerned, the occurrence of a case in the Kumaon hills, and another in Assam, two places nearly a thousand miles apart, is indicative of an enzootic area along the lower range of the Himalayas which may extend into Burma. Again the infection of Indian tigers recorded abroad suggests that the enzootic area is associated with the distribution of the wild Felidae. The geographical distribution of Paragonimiasis in countries such as surround India on the North and East, viz., China, Indo-China, Annam, Malay Peninsula, and Sumatra, increases possibilities of the dissemination of this affection in India on account of (1) adjacency, (2) the trans-Himalayan origin of rivers of Indian plains which may bring with them the two kinds of intermediaries namely snails and crustacea infested with *Paragonimus* cercariae and cysts, (3) the wide range of travel possessed by wild beasts, which may serve to scatter the lung-fluke eggs far and wide to be taken up by suitable intermediate hosts.

The known snail hosts are (1) nine species of the genus *Melania* (*Tiara*) in Japan, and (2) one species of *Ampullaria* in Venezuela. The old genus *Tiara* is represented by nine sub-genera in India. Out of these two species of the sub-genus *Acrostoma* and three of *Melanoides*, the former showed two kinds of cercariae per species, while the latter harboured four, two and seventeen different cercariae per species as listed by Sewell (1922). None of these cercariae described seem to approach the one described for *Paragonimus* in China (Faust, 1922). In view of this knowledge, three questions that remain to be solved are

(1) Are the above snail hosts likely to harbour cercariae other than those described by Sewell, including among them those of *Paragonimus*?

(2) Can the *Paragonimus* cercariae be harboured by other snails present in India?

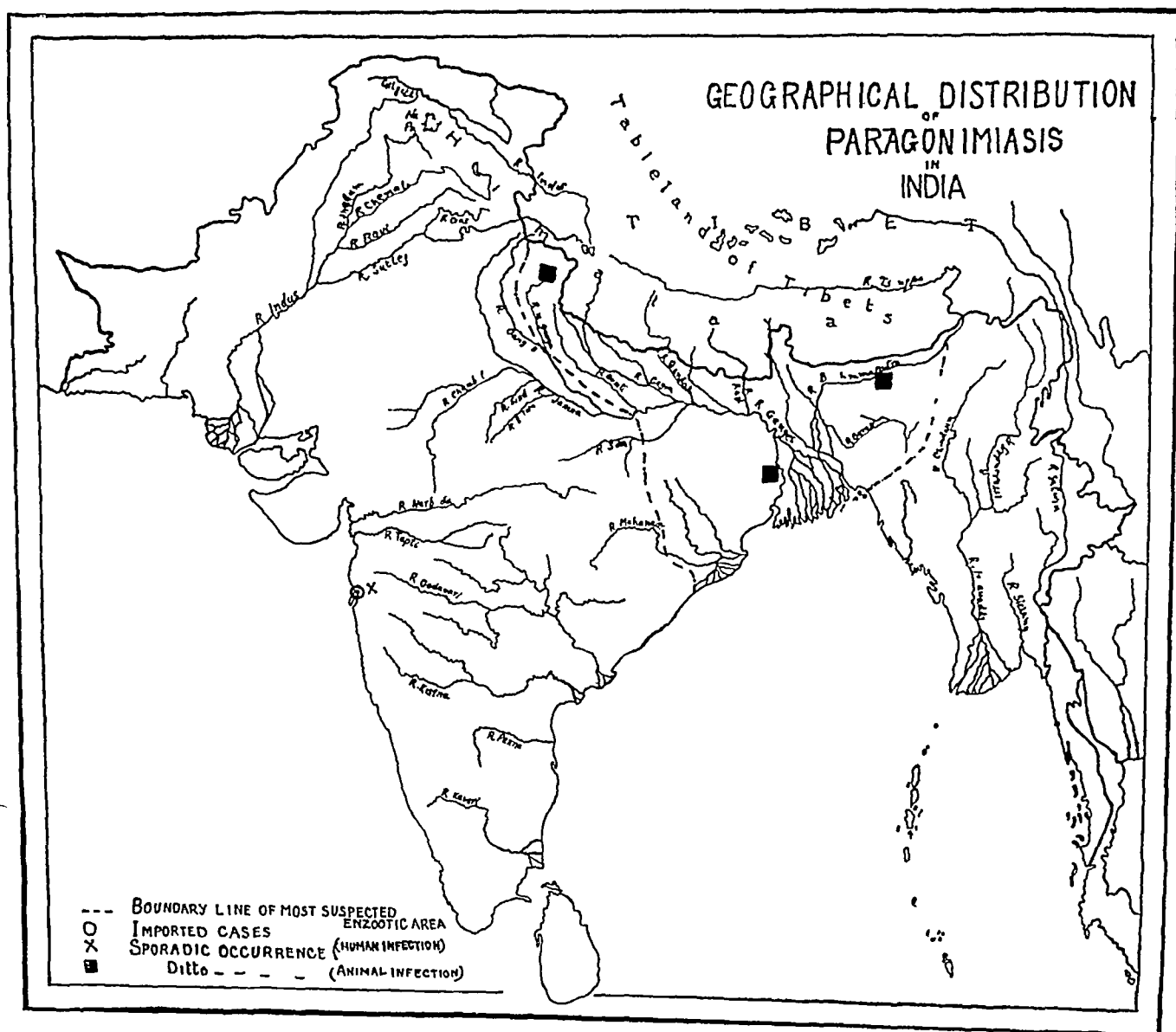
(3) In the event of the absence of a favourable field can the affection constantly pour into India in encysted form through the second intermediate hosts or crustacea?

The known crustacean hosts according to the Japanese workers, as cited by Baylis (1929) are three species of the genus *Potamon* covering as its synonyms the genera *Geothelphusa* and *Parathelphusa*, one species of the genus *Sesarma*, two species of the genus *Emocheir*, and three species again of the genus *Astacus*. In Venezuela the reported crustacean host belongs to the genus *Pseudothelphusa*. Among a large number of genera belonging to the family *Potamonidae*, Kemp (1924) records species of the genus *Parathelphusa*, mentioned above as a synonym of *Potamon*, in the collection of the Indian Museum, Calcutta. As for the other above named genera, further search appears necessary before their absence from India can be verified.

At and around Muktesai where infected *Paradoxurus grayi* was found, between an altitude of 5,000 to 7,500 feet, in a vicinity of about five miles radius in the

Kumaon hills, the writer's attempts to search for fresh-water snails in streams and springs were never successful during his stay of three years in that part of the country. Snails of the genera *Limnaea* and *Indoplanorbis* are present in Bhimtal

MAP



at a height of about 4,000 feet above sea-level. Cercariae from both these kinds of snails were studied, though not extensively, and it was noticed that *Indoplanorbis* harboured *Schistosomum* cercariae. This would account for the incidence of

schistosomiasis in equines, cattle and sheep at Muktesar detected by Montgomerie (1906) The cercariæ studied from *Limnæa* spp were apparently all already described by Sewell (1922) It is therefore noted with some interest that although *Paradoxurus grayi* the optimum host of *P. edwardsi* was found at Muktesar, yet the vicinity showed no signs of the presence of any intermediate hosts This fact is noteworthy for it takes us back to the possibility suggested above that wild animals can pick up the affection from some far off place may be, in the plains of India or from the trans-Himalayan plateau In either case a thorough search for human affection is suggested along the base of the Himalayan range including the Gangetic plain, the most suspected enzootic area, where at least some of both the necessary intermediate hosts are known to exist

Note—It is acknowledged that the facts of this note were gathered by the writer when he was employed at the Imperial Institute of Veterinary Research, Muktesar, during his stay there from June 1924 to July 1927 He has pleasure in according thanks to the Director of the said Institute for kindly permitting him to publish the above matter in his own name

The above Map marks the place of occurrence of *Paragonimus* spp and also the suspected enzootic area

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ACTION OF NARCOTINE ON THE GASTRO-INTESTINAL TRACT

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Of the alkaloids of opium, narcotine is second only to morphine as regards the quantity of alkaloid in the crude product. In the manufacture of morphine therefore a considerable amount of narcotine is produced and in order to see if this could be made use of in therapeutics, we made an investigation into its pharmacological action (Chopra, Mukerjee and Dikshit, 1930). The depressant action of the drug on non-striated muscles of the body was demonstrated and it was suggested that among other therapeutic applications of the drug, its sedative action on the gastro-intestinal system might be utilized. This study was therefore undertaken to determine in some details the action of narcotine on the gastro-intestinal tract.

Experiments were conducted on the amphibian and mammalian stomach and intestines. Mammalian experiments no doubt give more information about the possible action of the drug in human beings. The powerful depressant action of the anæsthetic in mammalian experiments both on the movements and the secretions of the gastro-intestinal tract is a disadvantage in ordinary methods. Experiments were therefore conducted to determine the action on animals without giving any anæsthesia.

Absorption —Introduction of the drug (0.1 g. per kilo of body-weight) through the gastric fistula of a cat will bring about symptoms of narcotine absorption like salivation and restlessness within about 14 to 20 minutes. A bigger dose may produce the action in lesser time but leads to toxic symptoms. Rectal injections of 0.1 g. per kilo doses, however, bring about the symptoms of absorption within about 7 minutes. The drug is therefore absorbed more rapidly from the colon.

An injection of the same dose (0.1 g. per kilo) given into the exteriorized loop of the small intestines of an animal will produce symptoms within about 10 minutes. It is evident from the foregoing experiments that the drug is absorbed fairly rapidly

from the smaller intestines and the colon. It can not be said with certainty, however, that the drug is absorbed from the stomach as well. The drug introduced into the stomach produces symptoms within about 14 minutes. Within this time it will have reached the smaller intestines and have produced symptoms from being absorbed from there. As will be shown later the drug has a marked influence on the emptying time of the stomach and it is retained in the stomach for a long time. Sufficient quantities, however, will be able to leave the stomach and enter the small intestine before the action on the pylorus is manifested. To study the absorption of the drug from the stomach, therefore, the following experiment was done. The abdominal cavity of guinea-pigs was opened under ether anæsthesia and the anterior wall of the stomach sutured in the wound after ligating the pylorus. The animals were allowed to recover from anæsthesia and the drug injected directly into the stomach cavity. Control experiments were done by performing the same operation but injecting normal saline instead of narcotine solution. The animals were watched for about six hours to see if any symptoms of absorption of the drug were manifested. As a rule no symptoms are seen. The animals generally die within 24 hours after the operation of ligation of the stomach. The stomach, small intestines and the colon are ligated separately and their contents examined for the presence of the alkaloid, for, as will be shown later, the drug, after absorption, is excreted through the bowels. It is found that the small intestines show a trace of the alkaloid while the colon is free from it. This shows that the drug is absorbed in very small quantities from the stomach if it is allowed to remain there for a sufficiently long time. Narcotine therefore is absorbed from all the three portions of the gastro-intestinal tract although the absorption from the stomach is negligible.

Excretion —Excretion of the drug was studied in rabbits. A dose of 50 mg per kilo was injected hypodermically in animals who were starved 24 hours previously and were given only water to drink. The animals were again starved after the injection and killed after about 24 hours. The stomach, small intestine and the colon were ligated separately and removed. The contents of each were washed with distilled water, acidulated with hydrochloric acid, filtered and tested with Mayer's reagent. It is found that the intestinal washings give a heavy precipitate while the washings from the stomach show only a slight haziness. Washings from the colon give a distinct precipitate though not so heavy as in the case of the small intestines. It can be surmised from the above experiments that the drug is excreted mostly through the small and large intestines and traces of it through the stomach. Of course in such methods it is quite possible for the stomach contents to empty themselves into the intestines and the intestinal into the colon. The results are, however, sufficient to conclude that the drug is excreted through the gastro-intestinal tract.

Movements of the stomach —The depressant action of narcotine on the unstriped muscles of the frog's stomach is seen markedly. There is sometimes a slight initial

stimulation of the muscle followed by a marked and complete inhibition of the movements of the fundal as well as the pyloric portion of the stomach. Isolated perfused muscle strips of the frog's stomach show a similarly marked depression of the tone of the muscle and inhibition of the automatic movements, after addition of narcotine so as to give a concentration of 1 in 5,000 or more.

Intravenous injections of narcotine produce a relaxation of the tone and inhibition of the movements of the stomach of animals like the cat and the dog. The movements were studied by fixing one end of the Jackson's enterograph to a point in the greater curvature of the stomach and pinning another point about one inch away and fixing it to a writing lever. The depression is very clearly seen though it is not so markedly evident as in the case of the frog because the muscle is already depressed by urethane which anæsthetic was generally used for these experiments.

Experiments were therefore conducted to see the effect of narcotine on the movements of the mammalian stomach without any anæsthesia. Cats were used for this purpose. A gastrotomy opening was made in the stomach of a cat by the ordinary method. A cone from the anterior wall of the stomach was pulled out, and the base of the cone stitched to the anterior abdominal wall. The apex was taken under the skin for a distance of about one inch, incised and stitched to a small cut in the skin. The main incision was closed and the wound allowed to heal. A rubber tube was inserted into the gastrotomy opening to prevent it from being closed. The animal recovers within about a week. Experiments in this series were conducted on cats operated about six months before. Plate XXXVIII, fig A, shows the action of narcotine in such an experiment. The animal was starved twelve hours before, a rubber balloon was inserted into the stomach and a rubber catheter inserted into the rectum to give the injection without disturbing the animal. A dose of 0.1 g per kilo was administered. It will be seen that the stomach movements are slightly increased followed by an inhibition and complete cessation. Recovery takes place after about one hour.

If the drug is administered directly into the stomach the inhibition of hunger contractions sets in immediately and lasts for about an hour (Plate XXXVIII, fig C). The hunger contractions of the stomach are inhibited in a number of ways and many times simple chewing of food-stuffs is sufficient to inhibit them. Similarly introduction of water, saline or any other fluid will cause a temporary inhibition of hunger contractions. The sudden cessation of hunger contractions after introduction of narcotine solutions is therefore not of much importance. The prolonged inhibition of contractions is, however, significant for it is not brought about by water or saline. Narcotine may therefore find some use as a drug which alienates hunger.

Narcotine, however, has another important action, that of causing a remarkable delay in the emptying time of the stomach.

The delay in the emptying time of the stomach can be demonstrated in both amphibian and mammalian stomachs. A cannula is inserted into the cardiac end of the frog's stomach through the oesophagus and another into the duodenum within two to four millimetres of the pylorus. The stomach is removed from the body and perfused with frog's Ringer at constant pressure. The outlet is measured by recording drops by a Condens's drop recorder. The rhythmic character of the flow can very well be demonstrated by this method. An addition of narcotine solution to the perfusate immediately slackens the flow which continues at a slow speed till the whole of the narcotine solution is washed out of the stomach. A similar experiment conducted on a cat *in situ* will show a marked slackening of the flow of fluid from the pyloric end of the stomach. Plate XXXIX, figs E and F, show the effect of narcotine on the outflow and inflow of the stomach of a cat in such an experiment.

The remarkable delay caused in the emptying time of the stomach can be more strikingly seen in radiographic experiments. The usual dose of narcotine is introduced into the stomach followed twenty minutes later by a barium meal. Skiagrams are taken every two hours to see the progress of the meal. The usual emptying time of the stomach of a cat is about two hours. After narcotine however, the stomach contains a considerable amount after six hours and traces of the meal are seen even after twenty-four hours (Plate XL). It appears therefore that narcotine like morphine produces a powerful spasm of the pylorus and causes a delay in the emptying time of the stomach for several hours. This is an important action of narcotine and may explain the gastro-intestinal disturbances caused by the drug after administration of small doses in human beings.

Movements of the intestines—The depressant action of narcotine on the unstriated muscle of the intestines has been already demonstrated (Chopra Mukerjee and Dikshit, 1930). In acute experiments conducted under urethane anaesthesia narcotine given intravenously relaxes the tone of the muscle and diminishes its rhythmic movements. Perfusion experiments with rabbits and kitten's intestines similarly show a relaxation of the tone and inhibition of the movements (Plate XXXVIII, figs B and D).

The same results are seen in the case of the amphibian intestines both in perfusion experiments as well as in experiments *in situ*. The depressant action of the drug in acute experiments therefore is quite marked and can easily be demonstrated. It remains to be seen, however, how far the therapeutic doses will be able to produce the action especially when given by the oral method. To study this dogs with modified Thiry fistula were used.

Under morphine-chloroform anaesthesia, the abdomen of a dog was opened by a median incision and a length of bowel about eight to ten inches in length was taken out of the wound. A lateral anastomosis was done and the ends of the loop were ligated by strong silkworm gut just near the anastomosis. The continuity

of the gut was thus established while a loop was isolated from the gut with its nervous and vascular supply intact. A small portion, about half an inch of the convex border of the loop was then stitched in the abdominal incision which is then closed. An opening is made in the bowel wall and the wound allowed to heal. After the opening, the alkaline juice of the intestines causes irritation of the abdominal wall but beyond this there is no other effect produced. The animal is ready for experiments after about three or four days. Injections can be given by hypodermic methods or introducing the drug directly into the loop of the intestine or through the rectum. A balloon is inserted into the lumen of the loop of the bowel and tracings taken in the usual way. In such experiments doses of narcotine as high as 0.1 g per kilo produce a depression of the movements of the intestines which is not very marked and which lasts only for a short time (Plate XXXIX, figs A and B). It appears therefore that under ordinary conditions when narcotine is given by the oral method it will be able to cause depression of intestinal movements only in big doses and only for a short time. The effect is more marked when the intestinal muscle is excited by administration of pilocarpine but still a fairly large dose is necessary to bring about the action of inhibition. When administered by the oral method therefore narcotine has only a feeble depressant action on the movements of the intestines.

Movements of the colon—The effect of narcotine on the movements of the colon is the same as its effect on the small intestine. The action, however, is seen less markedly on this portion of the gastro-intestinal tract. Perfusion experiments with portions of the rabbit's colon show a relaxation of the tone of the muscle after addition of solutions of narcotine giving concentrations like 1 in 50,000 and more. In cats under urethane anaesthesia, intravenous injections of narcotine show a similar effect. Movements of the rabbit's colon without anaesthesia were studied by introducing a small balloon into the rectum of a rabbit and recording the contractions by connecting it with a tambour. Injections were given into the ear vein. Plate XXXIX, fig D, shows the effect of narcotine in such an experiment. 25 mg of narcotine per kilo were injected into the ear vein. There is relaxation of the tone of the muscle and inhibition of the contractions.

Movements of the œsophagus—Like the other portions of the gastro-intestinal tract, the movements of the œsophageal muscle too are inhibited by narcotine. Plate XXXIX, fig C, shows the effect of the drug on the automatic movements of the frog's œsophagus. Addition of narcotine to the perfusate so as to give a concentration of 1 in 50,000 produces an immediate cessation of the automatic movements of the muscle.

Action on secretions of the gastro-intestinal tract

(1) *Salivary secretion*—In acute experiments with urethane anaesthesia, injections of even large quantities of narcotine do not produce any appreciable

change in the amount of salivary secretion In experiments without anæsthesia, however, doses of narcotine, when given into the stomach through the gastric fistula or into the colon through the rectum, cause salivation It can not be said with certainty whether the salivation is direct or indirect from the foregoing experiments It is, however, quite possible that the action of narcotine causing a powerful spasm of the pylorus may be responsible for causing nausea and this may in its turn cause an increase in the salivary secretion As will be seen later, administration of narcotine to human beings causes a definite sensation of nausea in comparatively small doses The sialogogue action of the drug is therefore more probably an indirect one when it is seen in animals after the administration of narcotine

(ii) *Gastric secretion* —In absence of a Pawlov's pouch the gastric secretion was studied through the opening of a gastric fistula in a cat By this method small changes in the amount of the secretion can not be demonstrated, but if the changes are sufficiently marked they can be estimated by measuring the amount of secretion directly It was found that there was no appreciable change in the amount of gastric secretion after hypodermic injections of narcotine given in sufficiently large doses

(iii) *Intestinal secretion* —In order to see if there was any increase or decrease in the amount of intestinal secretion after the administration of narcotine, a small rubber tube, with holes on all sides, was inserted into the opening of a Thiry's fistula in a dog, and the juice collected in a rubber bag The amount of secretion at hourly intervals was measured which served as controls The injections were then given hypodermically In such experiments, even large doses of narcotine do not show any marked variation in the amount of secretion from the controls done It appears therefore that the drug has not got any marked action on the secretion of the intestinal juice

Action on enzymes

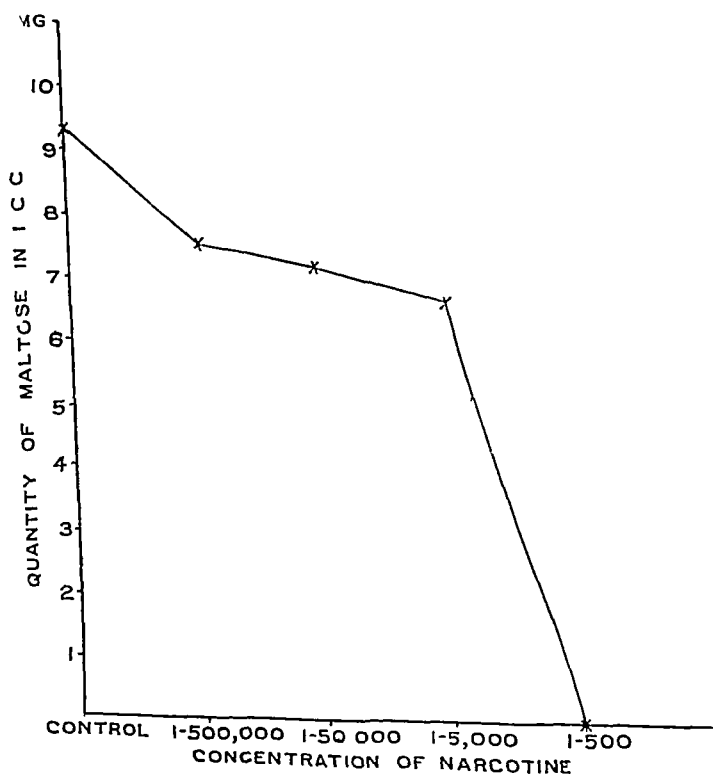
(i) *Proteolytic digestion* —I am indebted to Dr B P Mukerji of the Calcutta School of Tropical Medicine and Hygiene, for having carried out experiments to see the action of narcotine on the proteolytic enzymes Benger's Liq Pancreaticus with twice its volume of one per cent sodium carbonate solution, shaken up well with a little *Fel bovinum*, was used as the digesting agent Digestion on protein was tested by the action of this artificial pancreatic juice on unstained fibrin and noting changes in peptone formation as estimated by biuret reaction To several tubes containing the artificial pancreatic juice quantities of narcotine were added so as to give concentrations of the drug varying between 1 in 200,000 to 1 in 1,000 Fibrin was added to each tube and all the tubes incubated for three hours It was found that even strong concentrations of narcotine did not interfere much with the digestion Narcotine appears to have no marked action on the proteolytic digestion

(ii) *Peptic digestion* —Carminc fibrin method of peptic digestion was used To several tubes containing pepsin with 0.2 per cent hydrochloric acid, different

quantities of narcotine were added so as to give concentrations varying from 1 in 100,000 to 1 in 1,000. Small bits of stained fibrin were then added to each and the tubes incubated. The degree of digestion was judged by noticing the amount of dye liberated from the digested fibrin. It is found that although the digestion of fibrin is comparatively less with higher concentrations of narcotine, with these concentrations the digestion is not interfered with to any marked extent. Narcotine therefore has an inhibitory action on peptic digestion which is very feeble.

(iii) *Amylolytic digestion* — To study the action of narcotine on the digestion of starch, saliva was used as the digesting agent. Equal quantities of saliva freely diluted and a 3 per cent solution of starch were incubated for fifteen minutes after adding narcotine to give various concentrations between 1 in 500,000 and 1 in 500. The amount of maltose formed in the different tubes was then estimated by using Benedict's reagent. The following Graph gives the results obtained in such an experiment —

GRAPH



Horizontal line represents the concentration of narcotine in the tubes, while the vertical represents the amount (in mg) of maltose in those tubes. The tubes contained equal quantities of starch and saliva and were incubated for fifteen minutes.

It will be seen from the Graph that with 1 in 500 concentration of narcotine there is a complete inhibition of salivary digestion while with higher concentrations the digestion is only slightly inhibited. The difference in different concentrations between 1 in 500,000 and 1 in 5,000 is only slight. Narcotine therefore has, only in strong concentrations, a marked inhibitory influence on the salivary digestion.

Symptoms in human beings—The effects produced by the drug were seen by taking a small dose of narcotine on an empty stomach. There was a slight nausea and vertigo especially on moving the head from side to side. Disinclination to take food persisted for about four hours. There was no tendency to constipation after 5-grain doses. The drug was given in 5-grain doses three times a day to an asthmatic patient. He complained of about the same symptoms—nausea, giddiness, especially when trying to sit up in bed, and disinclination to take food. There was a slight tendency to constipation. Similar symptoms were produced in other healthy individuals who volunteered to take the drug and report the effects produced.

DISCUSSION

It will be seen from the experimental data given above that the important actions of narcotine on the gastro-intestinal tract are the following. It produces a powerful spasm of the pylorus and remarkably delays the emptying time of the stomach. It has a depressant action on the plain muscles and inhibits the movements of the gastro-intestinal tract, and thirdly it slightly interferes with the different digestive processes of the body. Out of these three important actions, the second, namely, its sedative effect on the gastro-intestinal tract offers itself as a possible reason for its employment in therapeutics. It will be seen, however, that the depression is seen only when sufficiently large doses are given and such doses are very likely to interfere with digestion in a deleterious way. It was thought that the depressant action of the drug on the movements of the intestines may be used to counteract the griping associated with some purgatives. If sufficiently large doses are given the drug may bring about the desired effect of reducing griping, but the other side effects produced contra-indicate its use as such. 5-grain doses given three times a day only show a tendency to constipation. It can not be said with certainty that in such doses the depressant action of the drug on the muscles of the bowels is manifested, for the tendency may be due to the spasm of the pylorus which itself may be sufficient to produce constipation. It is therefore evident that if the drug is at all to be used to allay griping it must be given in larger doses than 5 grains t. d. s. Such doses are sure to upset the digestion to a great extent and the effects will last for some time. It is not therefore likely that narcotine will find a use as a corrective for purgatives. The inhibitory influence of narcotine on the hunger contractions of the stomach indicate its use as a drug which relieves hunger. It may be used as such but clinical trials on a larger scale alone will prove whether it is of use to diminish hunger. The undesirable side effects, however, may

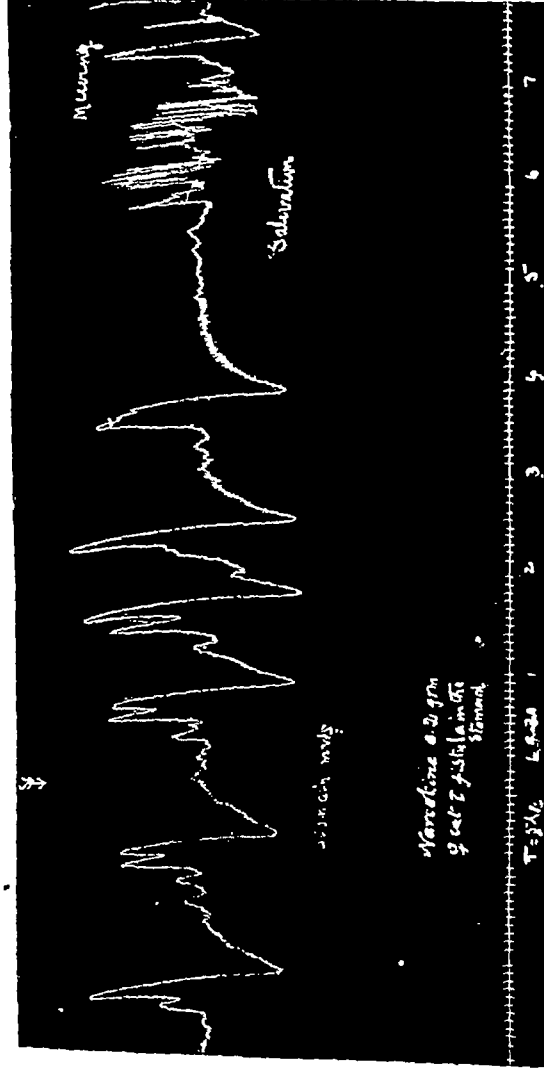


Fig A

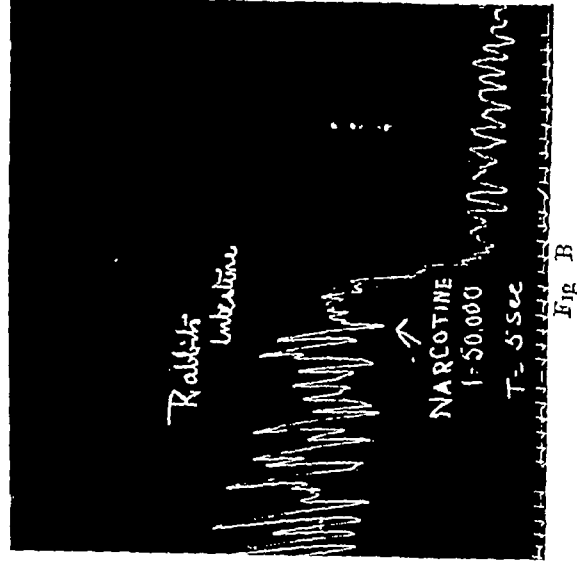


Fig B

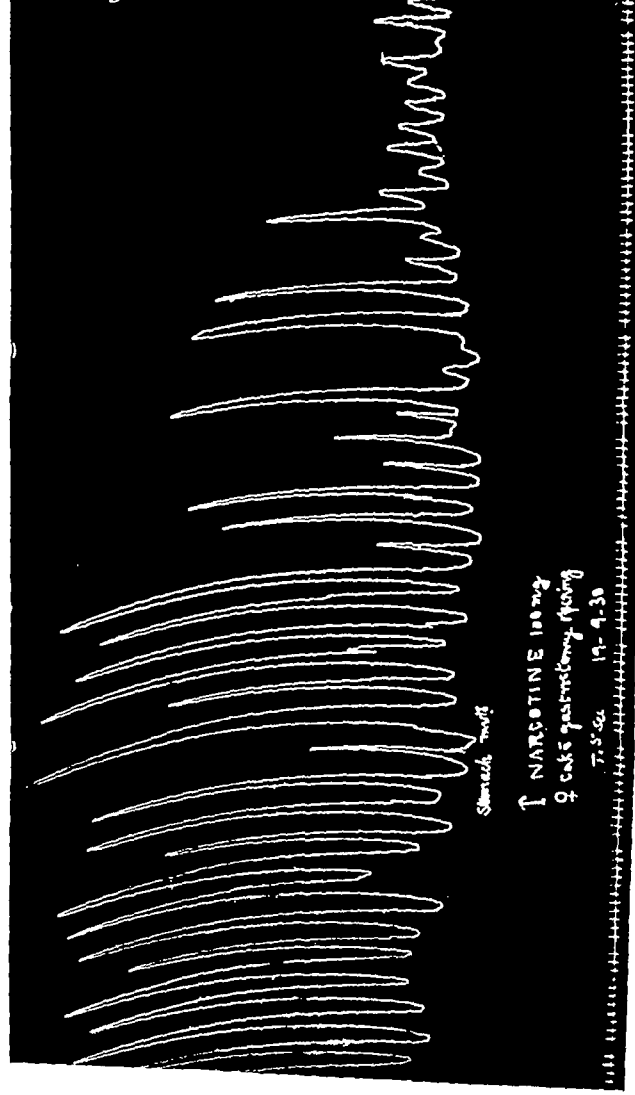


Fig C

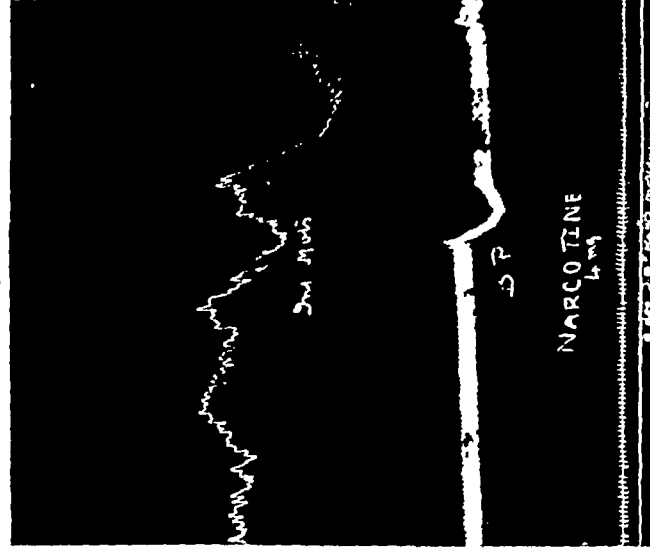


Fig D

Fig A Shows the stomach movements At arrow mark narcotine given per rectum Note slight increase followed by decrease of movements
 " B Shows movements of isolated rabbit's intestines Narcotine at arrow mark Note the relaxation of the tone and diminution of movements
 " C Shows hunger contractions of the stomach Note the inhibition of contraction after introduction of narcotine into the stomach

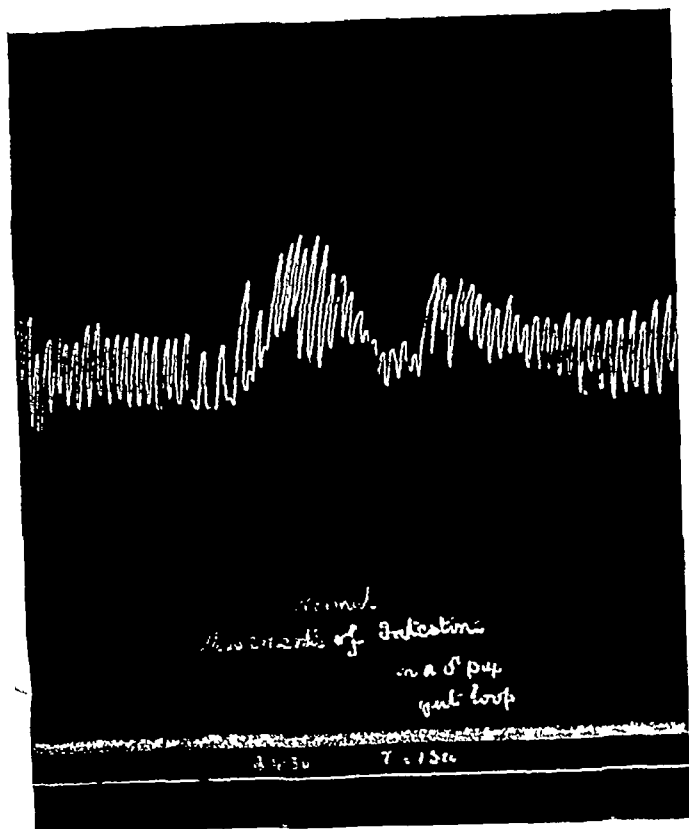


Fig A

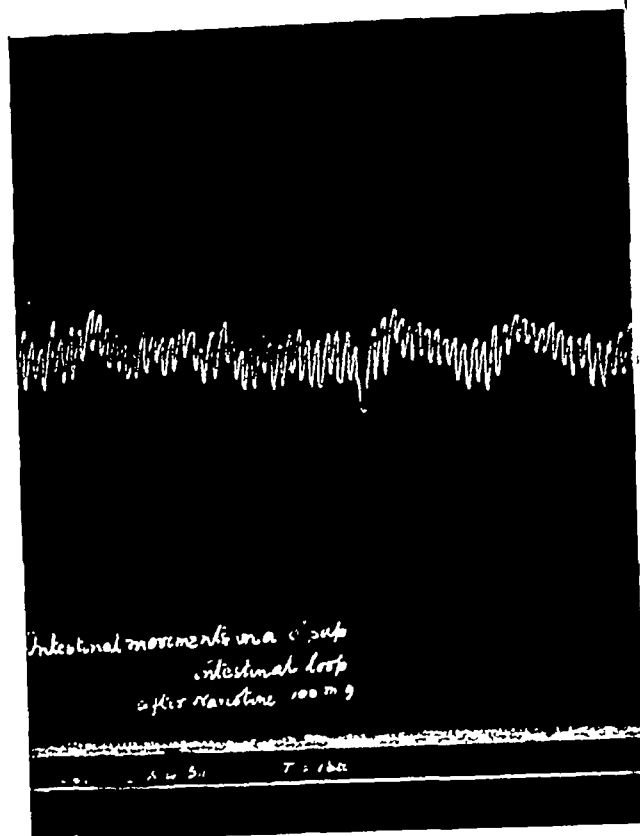


Fig B

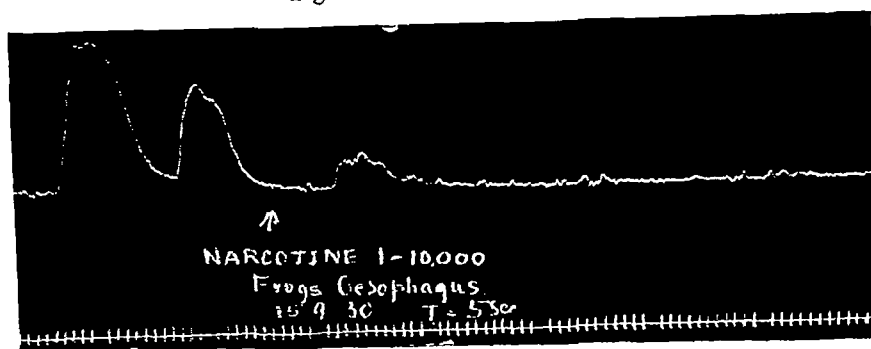


Fig C

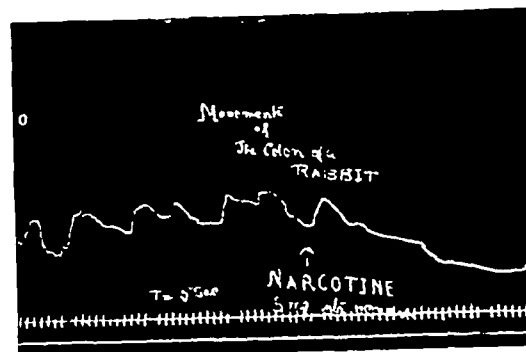


Fig D

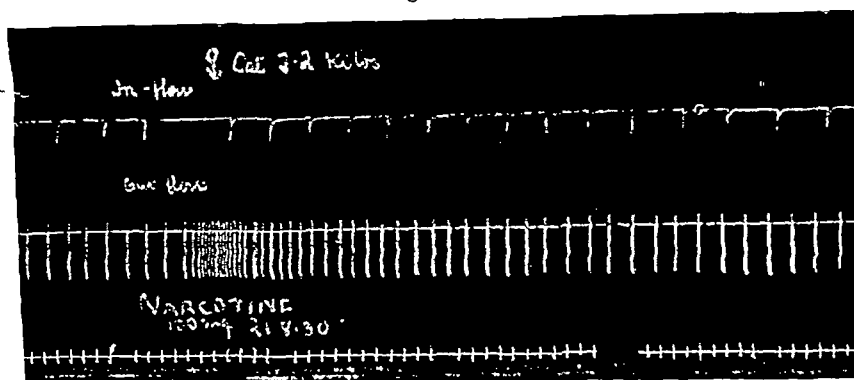


Fig E

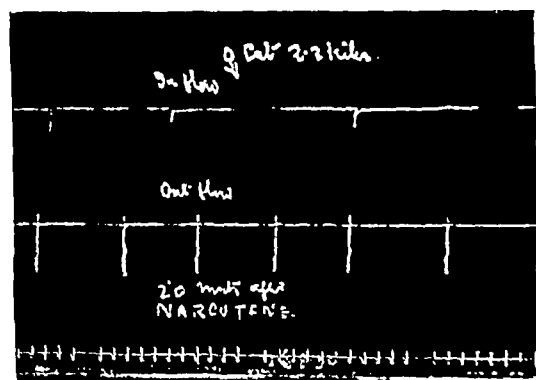


Fig F

- Fig A Shows the intestinal movements of a pup as they occur normally
- „ B Shows the same movements after administration of 100 mg of narcotine to the pup
- „ C Shows the effect of narcotine in 1 in 10,000 dilutions on the isolated strip of a frog's oesophagus The drug was added at the arrow mark Note the complete cessation of automatic movements
- „ D Shows the movements of the colon of a rabbit Narcotine given at arrow mark. Note the inhibition of movements and relaxation of the tone of the muscle Intravenous injection given
- „ E Upper tracing inflow lower tracing out flow of the stomach of a cat Narcotine given at the notch Acceleration of flow due to pressure of injection and subsequent recovery
- „ F Same as Fig E after 20 minutes Note the marked reduction of the flow



Fig A



Fig B



Fig C

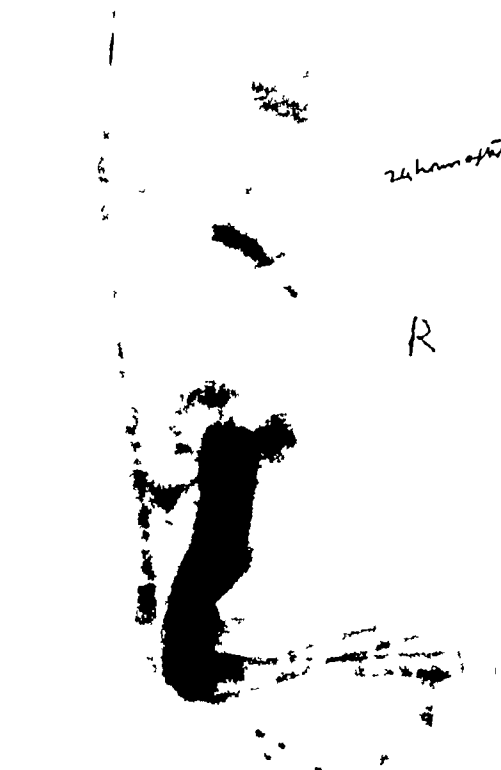


Fig D

Fig A Shows the shadow of a barium meal in a cat which was given narcotine about half an hour before the meal was given The photo was taken about 10 minutes after the meal
 B Taken two hours after the barium meal Note that a considerable amount is still present in the stomach
 C Taken six hours after the barium meal Note the presence of part of the meal in the stomach
 D Taken twenty-four hours after the meal Note that a trace is still left in the stomach

contra-indicate its use. It will be seen therefore that narcotine does not promise to be a useful drug so far as its action on the gastro-intestinal tract is concerned. It can not be used with much advantage in therapeutics, to treat cases of gastro-intestinal diseases.

SUMMARY AND CONCLUSIONS

(1) Narcotine is absorbed through intestines and to a certain extent from the stomach as well.

(2) It causes a marked delay in the emptying time of the stomach and inhibits the hunger contractions.

(3) In sufficiently large doses it causes a relaxation of the tone and inhibition of the movements of the intestines.

(4) Its effect on the different secretions of the gastro-intestinal tract is not marked. The enzyme actions are slightly inhibited.

(5) Symptoms in man are described.

(6) Narcotine is not likely to prove of much use in therapeutics as far as its action on the gastro-intestinal tract is concerned.

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PHARMACOLOGY OF SALTS OF FATTY ACIDS OF CHAULMOOGRA OIL

Part I.

ALEPOL

BY

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[Received for publication, October 9, 1931]

CHAULMOOGRA oil has been used in the treatment of leprosy for a very long time in India. It was taken internally as well as applied externally to leprosy lesions. The irritant nature of the oil, however, prohibited its use in sufficiently large quantities because it could not be tolerated. Its use therefore was limited till the method of giving it by intramuscular and hypodermic injection was brought into prominence.

Power and his co-workers (1904) determined the chemical nature of the important constituents of the oil and showed that it consists of glycerides of a group of unsaturated fatty acids which are optically active and are very peculiar in having a closed five carbon ring. Hydnocarpic and chaulmoogric acids were shown to be the two important acids of the oil and it is believed that the former is more potent than the latter in the treatment of leprosy. In 1913 Heiser reported successful treatment of leprosy by giving the oil by intramuscular injections. This was a great step in advance for the drug could now be given in much larger doses. The irritant nature of the oil, however, is a drawback associated with this method as it produces pain and sometimes leads to necrosis at the site of injection. If, however, the oil is prepared after due precautions as advised by Muir the irritant action of the oil is considerably lessened. In 1916 Rogers prepared sodium salts of the mixed acids and administered them in cases of leprosy with brilliant results. Roger's

success clearly established the value of chaulmoogra salts in the treatment of leprosy and the method was used very extensively with gratifying results. These salts too possess the irritant action of the oil and this constitutes a great disadvantage especially if the salts are to be administered by the intravenous method. Rogers (1916 *a* and *b*) is inclined to attribute the pain to the presence of palmitic acid. Working subsequently he expressed doubts if the chaulmoogric and palmitic fraction is of real value. The remaining acids which in his case has a melting point between 37 and 40 degrees gave soluble sodium salts and he advocated the use of these salts in 2 to 3 per cent solutions intravenously. Martindale (1922) working on about the same lines fractionated the acids of chaulmoogra oil in two groups and advised their use as sodium salts. The first termed Sodium Chaulmoograte 'A' comprises the salts of higher acids with relatively high melting points. The second termed Sodium Chaulmoograte 'C' comprises the lower acids with relatively low melting points. It contains the lower homologues with combined melting point of 25°C approximately. In a previous communication (1931) we have reported successful treatment of leprosy with one of the sodium salts of a lower melting point fatty acid of hydnocarpus oil named by the manufacturers (Burroughs Wellcome & Co) as 'Alepil'. It is the object of this study to compare the pharmacological actions and therapeutic uses of soaps prepared from the whole oil and those prepared from certain fractions of the oil especially the lower ones possessing a low melting point. A study of the pharmacological action of Alepil is given in this paper.

EXPERIMENTAL

Experiments were conducted on cats, dogs and rabbits with and without anaesthesia. Guinea-pigs and frogs were also used in some experiments to compare the actions as for example in toxicity tests. Urethane anaesthesia was generally used for cats and rabbits and in dogs it was supplemented by morphine. In experiments without anaesthesia when an operated animal was used, care was taken to see that at least one week had elapsed between the operation and the experiments. In most of the experiments animals operated about three months previously were used but some in which the operation was done only a week before are also included in these experiments. The operated animals were trained to lie down on the table during the experiment. Experiments were done on all the three species to see if there was any difference in the response. It was found that the nature of response in all the animals was the same.

TOXICITY

Toxicity tests were done in frogs, guinea-pigs, rabbits, cats and dogs with and without anaesthesia and in different concentrations of the solutions.

(i) *Frogs* —When injections are given in the anterior lymph sac frogs tolerate a dose of 0.3 mg per gramme of body-weight, associated, however, with very severe symptoms and a dose of 0.4 mg is as a rule fatal. Post-mortem examination after a fatal dose shows a marked depression of all the unstriped muscles of the body if the examination is done immediately after death. The left heart is markedly contracted and the right dilated.

(ii) *Guinea-pigs* —Toxicity in these animals was tried by hypodermic injections of Alepol. When administered by this method the average toxicity works up to 0.45 grammes per kilo of body-weight. Animals which are given gradually increasing doses of the drug show a number of hæmorrhagic patches especially under the skin and in the lungs. Post-mortem examination of animals after a fatal dose shows a contracted left heart, a dilated right heart and patches of hæmorrhages in several places. The liver shows fatty infiltration in a majority of cases. Kidneys are hyperæmic but in many cases signs of inflammation were wanting.

(iii) *Rabbits* —When strong concentrations like 20 per cent are injected intravenously in rabbits they tolerate a dose of about 0.025 g per kilo of body-weight associated with very severe symptoms. Patches of hæmorrhage in various places under the skin are seen after 24 hours. If in an animal like this a very small dose is injected the next day this dose is sufficient to kill the animal. An average dose of 0.027 g per kilo is as a rule fatal in rabbits when 20 per cent strengths are used. With 7 per cent strengths, however, an intravenous dose of 0.055 g per kilo can be tolerated with very severe symptoms and with more dilute solutions like 3 per cent the fatal dose is somewhere between 0.06 and 0.065 g per kilo. The toxicity is dependent upon the rate of injection. In all the above experiments the rate of injections was the same. It will be seen therefore that the toxicity of Alepol markedly increases with the increase in the concentration of the solution. The difference between 20 and 7 per cent strengths is twice as much. With toxic doses the animal dies with convulsions. Post-mortem examination in these animals shows about the same changes as are seen in the guinea-pig. The left heart is contracted while the right is dilated.

(iv) *Dogs* —Toxicity in dogs was tried in animals under morphine-urethane anæsthesia. A 3 per cent solution was slowly introduced into the femoral vein through a cannula. The average toxicity in these animals when determined by this method is about 0.3 g per kilo of body-weight.

(v) *Cats* —To determine the toxicity of Alepol in cats both intravenous and hypodermic injections were given. The former were given in animals under urethane anæsthesia while the latter were given to animals without any anæsthesia. When given by the intravenous method the toxicity is about the same as in dogs, that is about 0.3 g per kilo of body-weight. Hypodermically 0.4 g per kilo are tolerated associated, however, with loss of weight and appetite. The fatal dose will be considerably higher than this dose.

The following table gives a comparison between the toxicity of Alepol and Martindale's 'C' preparation, both of which belong to the lower fatty acid group of chaulmoogra oil and have a low melting point —

TABLE I

Shows the comparative toxicity between Alepol and Martindale's 'C' preparation

Animal	Strength of solution per cent	DOSE IN GRAMMS PER KILO		REMARKS
		Alepol	'C'	
Rabbit	20 I V	0.025	0.10	Tolerated
Rabbit	2 I V	0.050	0.05	Marked symptom
Rabbit	2 I V	0.065	0.12	Death
Cat (U)	20 I V	0.20	0.10	Survived
Cat (U)	20 I V	0.30	0.18	Death
Cat	20 H D	0.10	0.30	Loss of weight

I V = Intravenous injection, H D = Hypodermic injection, (U) = Urethane anaesthesia

It will be seen from the table given above that the toxicity of Alepol compares favourably with that of Martindale's 'C' preparation. The toxicity in rabbits, however, shows marked difference. In 20 per cent strength Martindale's 'C' can be tolerated in 0.1 g doses while 0.027 g of Alepol in the same strength are fatal. With more dilute concentrations the difference is less. In cats Alepol is uniformly less toxic than the other preparation.

ACTION ON TUBERCLE BACILLI

Walker and Sweeny (1920) have demonstrated the remarkable toxic action of chaulmoogra salts on the growth of tubercle bacilli. They find that the sodium salts of the fatty acids of the chaulmoogra oil arrest the growth of the acid-fast bacteria in dilutions of 1 in 1,000,000, and kill them in 1 in 100,000. This shows that they are about 100 times as active as phenol. This action, however, is seen only on

acid-fast bacteria Experiments were done to see the action of Alepol also on the growth of tubercle bacilli The results are given in the following table —

TABLE II

Shows the growth of tubercle bacillus in a glucose broth culture medium containing various concentrations of Alepol

Control	1 in 1,000,000	1 in 500,000	1 in 200,000	1 in 100,000	1 in 50,000	1 in 20,000	1 in 10,000	1 in 5,000	1 in 1,000
++	+	+	—	—	—	—	—	—	—

+ = Growth, — = No growth

A naked-eye examination of the culture tubes was made every week and it was found that even in a dilution of 1 in 1,000,000 there was no growth up to two weeks. A microscopic examination at the end of three months showed an arrest of growth in concentrations of 1 in 200,000. The control showed very good growth while the growth in 1 in 1,000,000 and 1 in 500,000 dilutions was not very marked. Experiments to determine the strength necessary to kill the organisms are in progress and will be reported later. It will, however, be seen that Alepol too possesses the remarkable toxic action on acid-fast bacteria that is characteristic of the other salts of chaulmoogra.

ACTION ON CERTAIN HELMINTHS

(i) *Filaria* —The specific toxic action on the tubercle bacilli exerted by the derivatives of the chaulmoogra oil appears to be due to their solvent action on the waxy envelop of these bacteria. It was therefore thought that the drug may be useful in filariasis as well. At the suggestion of, and in collaboration with, Dr C G Pandit of the King Institute of Preventive Medicine, Gundy, Madras, the effect of Alepol injections in the filariasis of crows was studied. When intravenous injections of Alepol are administered to crows, a marked reduction of the number of the microfilaria in the peripheral blood is seen within twenty-four hours. The effect, however, is not lasting and some time later the count again rises. The reduction in the count immediately after the injection is, however, very marked. In vitro experiments with microfilariae in the human blood did not show a marked susceptibility of microfilariae to Alepol. If high concentrations like 1 in 10,000 are brought

in contact with a drop of human blood containing microfilariae and examined under a microscope it is found that the parasites are quite active after a contact of about two hours. With higher concentrations like 1 in 5,000 their movements become markedly sluggish and slow after about one hour's contact, but even after 8 hours signs of life are still present. Work in connection with the treatment of filariasis with injections of Alepol in human beings is in progress.

(v) *Tapeworms*—Alepol appears to have a toxic action on some of the intestinal worms. The round and thread worms do not appear to be affected by even strong concentrations of the drug. The tapeworm however shows a distinct effect with comparatively low strengths. The following table shows results in one of the experiments on the *Tenæ* of cats *T serrata*—

TABLE III

Shows the effect of different concentrations of Alepol on the movements of T serrata of cats

Concentration	1-5	1-10	1-100	1-1,000	1-10,000	1-100,000
Time in minutes to render the worm motionless *	1†	1†	$\frac{1}{2}$	2	30	60

* Control with Locke's solution—Worm active after 24 hours † Immediate

In experiments like the one given above segments of the same worm of approximately the same length were used. Although the worm loses all movements, if it is transferred to Locke's solution the movements are regained in a short time. Concentrations as high as 1 in 10 even do not completely kill the segments of the worm after about seven minutes contact.

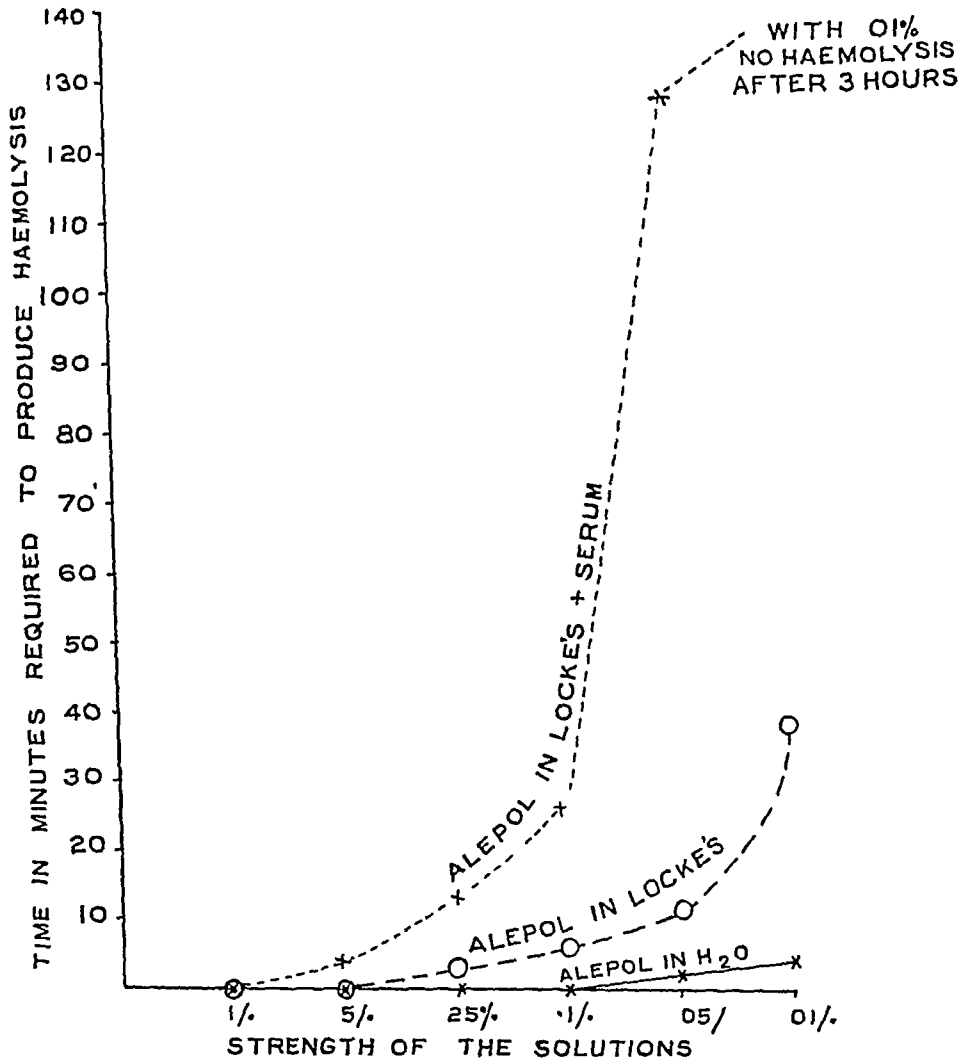
ACTION ON BLOOD

Being a soap, Alepol exerts a very marked hæmolytic action on the red blood corpuscles. If in a series of test-tubes containing different concentrations of Alepol varying between 10 per cent and 0.01 per cent, a few washed red blood corpuscles are added, the remarkable hæmolytic action of the solution is immediately seen. With the higher concentrations the red blood corpuscles disappear immediately giving a darkish tint to the solution. Similarly the disintegration of the red blood cell can be well seen under the microscope if different strengths of Alepol are brought in contact with them under the field. Control experiments with solutions of ordinary soaps show that soaps of chaulmoogra oil do not possess any greater hæmolytic action. The degree of hæmolysis produced by Alepol and other soaps of chaulmoogra is about the same as that produced by ordinary washing soaps.

This hæmolytic action of Alepol can however be considerably lessened if the solution of the soap is made in Locke's solution instead of distilled water

GRAPH

Shows the hæmolytic curves of Alepol dissolved in water, Locke's solution, and in the presence of serum



Alepol in Locke's plus serum + + + +
 Alepol in Locke's solution o — o — o — o
 Alepol in distilled water x — x — x — x
 Horizontal line indicates the strength of solution
 Vertical line gives time in minutes required to produce hæmolysis

Serum is now well known to be an agent which markedly affects the hæmolytic action of a number of substances It also reduces the hæmolytic effect of

Alepol The action of serum in reducing the hæmolytic effect of Alepol is illustrated in the following experiments. Two sets of tubes containing equal quantities of various strengths of Alepol are arranged on a rack. To one of the sets a few drops of serum are added. Equal amount of washed red blood corpuscles are then added to all the tubes and the time required completely to hæmolyse the red blood corpuscles noted. It is found that the set containing serum shows a much retarded hæmolytic action compared with the one containing no serum. This property of serum can be considerably enhanced if instead of the ordinary solution of Alepol, one prepared in Locke's solution is used. Both Locke's solution as well as serum retard the hæmolytic effect. The combination of the two, however, is very much superior. The above Graph shows the comparative hæmolytic action of Alepol dissolved in distilled water, dissolved in Locke's solution and dissolved in Locke's solution together with a small quantity of serum.

It will be seen from the Graph given above that Alepol dissolved in distilled water has much more hæmolytic action than Alepol dissolved in Locke's. A combination of Locke's and serum is remarkably efficacious in diminishing the hæmolytic action of the soap.

LOCAL ACTION

Applied locally to the skin Alepol behaves like ordinary soap, it has no irritant action whatever even when applied in strong concentrations. On mucous membranes, like the conjunctiva, however, it produces a marked irritant effect. A 3 per cent solution in distilled water instilled into the eye of a rabbit causes a marked local reaction resulting in intense hyperæmia and irritation. The animal tries to brush off the solution from its eyes. A 1 per cent solution also causes a local reaction and the eye becomes congested in about ten minutes.

Hypodermic injections in cats and rabbits show inflammatory changes within twenty-four hours if 3 per cent strengths are used. The effects, however, pass off after a short time. Intramuscular injections are better borne than hypodermic injections. With 5 per cent strengths given hypodermically, a fairly marked local reaction is produced and strengths higher than this produce more severe results. A 10 per cent solution leads to necrosis. The intensity of local reaction can, however, be diminished by dissolving Alepol in Locke's solution instead of distilled water. A 5 per cent solution in Locke's given hypodermically produces less local reaction than one in distilled water. The severity of the inflammation can still be lowered if a few drops of serum are added to Alepol dissolved in Locke's solution.

Intravenous injections of 1 per cent solutions prepared in distilled water produce obliteration of the vein after one or two injections, in rabbits. There is no such immediate obliteration if a solution prepared in Locke's is used. Addition of serum will still reduce the local reaction on the vessel endothelium and a large

number of injections can be given in the same vein. This property of serum in reducing the severity of local reaction is made use of in Muir's (1927) technique of withdrawing blood in the syringe, mixing and then injecting the mixture in the vein. In our clinical work it has been found that a solution of Alepol in Locke's given by Muir's technique permits a number of injections to be given in the same vein and the problem of obliterative endarteritis becomes far less important. Solution of Alepol in Locke's when given by injections intravenously without following Muir's technique does not obliterate the veins so readily and a number of injections can be given in the same vein.

ACTION ON THE CIRCULATORY SYSTEM

Intravenous injections of Alepol produce a fall of blood-pressure which is not very marked and which lasts only for a short time (Plate XLII, figs A, B and C). The fall is probably due to the direct action of the drug on the myocardium producing a depression and consequent lessened output of the heart. This action of Alepol can be demonstrated *in situ* as well as in perfusion experiments with mammalian and amphibian hearts. Plate XLI, fig A, shows the effect of a large dose of Alepol (30 mg per kilo), given intravenously in a cat, on the contractions of the heart. Both the auricle and the ventricle are depressed but the effect produced on the auricle is more marked than that on the ventricle. With smaller doses like 15 mg per kilo the depression is much less marked and lasts for a very short time only (Plate XLI, fig B). With still smaller doses like 5 mg per kilo no depressant action on the heart is seen.

Perfusion experiments with mammalian and amphibian hearts show a depression after sufficiently strong concentrations of Alepol. Plate XLI, fig D, shows the effect of Alepol on a perfused kitten's heart when added to the perfusate so as to give a concentration of 1 in 10,000. There is at first a transitory depression followed by recovery and this is followed by a uniform prolonged depression. A certain amount of irregularity is also present. With lower strengths the depression is still present but the irregularity is not evident. The degree of depression varies directly with the strength of the solution and with concentrations of Alepol less than 1 in 50,000 the depressant action is not at all marked. Amphibian hearts show about the same action as the mammalian hearts. In amphibian hearts, however, the initial depression is as a rule followed by a stimulation of the heart and this is later on followed by a depression which is directly proportional to the dose of Alepol given. The higher the concentration of the drug the greater is the depression of the heart produced.

The volumes of intra-abdominal organs do not show any marked variation after injections of Alepol. Sometimes an increase in the volume of the intestines is seen (Plate XLII, fig C), while the spleen may show a slight diminution together with an increase in the automatic contractions of the organ (Plate XLII, fig A).

ACTION ON THE RESPIRATORY SYSTEM

Small doses of Alepol given intravenously in rabbits without any anæsthesia produce a well marked stimulation of respiration. The same effect is observed when a similar dose is given to an animal under anæsthesia. Plate XLII, fig B, shows the stimulant action on respiration on a cat under urethane anæsthesia. The action perhaps might be due to the alkalinity of the solution only.

The bronchioles are dilated with smaller concentrations and constricted with larger ones. If the lungs of a cat are perfused with warm oxygenated Locke's solution through the pulmonary artery and the resistance to fixed quantities of air of artificial respiration recorded by a tambour, the effect of Alepol on the bronchioles can be well seen. Plate XLI, fig C, shows the effect of strong concentration of Alepol on the bronchioles of a cat. A concentration of 1 in 1,000 of Alepol was perfused through the lungs. It will be seen that this concentration produces a constriction of the bronchioles. After an intravenous injection of Alepol the changes in the calibre of the bronchiole will depend upon the dose given. A smaller dose will cause a dilation and a bigger will lead to constriction of the bronchioles.

ACTION ON THE GENITO-URINARY SYSTEM

Secretion of urine is not affected to any appreciable extent by administration of Alepol. If the flow of urine is measured in an anæsthetized animal before and after the administration of Alepol it is found that the rate of secretion remains to a very large extent unchanged. Doses varying between 5 to 20 mg per kilo do not affect the secretion. With larger doses a decrease in the rate of the secretion is seen. This is most probably dependent on changes in the blood pressure only for the effect passes off as soon as the blood-pressure resumes its normal level.

Movements of the uterus *in situ* are not affected by even large doses of Alepol. Plate XLII, fig B, shows the effect of an intravenous injection of Alepol in a non-pregnant cat. It will be seen that the tone of the muscle remains unchanged. In perfusion experiments concentrations varying between 1 in 150,000 and 1 in 50,000 show a tonic contraction of the uterus while stronger concentrations like 1 in 10,000 produce a relaxation. (Plate XLIII, figs C and D)

ACTION ON THE GASTRO-INTESTINAL SYSTEM

Alepol has got a peculiar soapy taste when applied to the tongue. It causes a slight salivation and frothing when a small quantity is introduced into the mouth. When 10 c c of a 5 per cent solution are introduced into the stomach of a dog by a stomach tube, the irritant action of the drug on the mucous membranes of the stomach leads to emesis within about 20 minutes. The emesis is due to local action and is not central as shown by the fact that much larger doses are tolerated by hypodermic injections without producing any symptoms of vomiting.

The effect of Alepol on the movements of the stomach of an unanæsthetized animal when given in subemetic doses is shown in Plate XLIII, fig E. A small balloon was introduced into the stomach of a cat through a gastric fistula and the records of the movements of the stomach were taken by connecting the balloon to a tambour. The animal was operated upon about eight weeks before. The drug was introduced into the stomach by a fine catheter introduced through the gastric opening. It will be seen from the figure that Alepol causes an increase in the tone of the muscle and the automatic movements are abolished. The tonic contraction is maintained for more than one hour and after this period the automatic movements gradually return to normal. Repeated oral administration of small quantities of Alepol leads to a chronic gastritis accompanied with anorexia and loss of weight.

Movements of the intestines are not affected to any appreciable extent by intravenous doses of Alepol varying from 10 to 20 mg per kilo of body-weight. In an anæsthetized animal the tone and the amplitude of contractions remains about the same after intravenous administration of the drug (Plate XLII, fig A). The effect of Alepol on the movements of the intestines of an animal without anæsthesia were studied in dogs with Thiry's fistula. An animal operated about one week before was used for the purpose. The wound had healed by this time and beyond the inflamed condition of the skin due to the irritant action of the intestinal juice the animal was in a perfectly healthy condition. It will be seen from Plate XLIII, figs A and B, that after a dose of 100 mg per kilo the amplitude of contractions of the intestinal muscle is lessened to a certain extent. This action is due to the direct depressant action of the drug on the unstriated muscles of the bowels. Perfusion experiments with rabbit's and cat's intestines show an increase in the tone of the muscle with high dilutions like 1 in 150,000 but with higher concentrations like 1 in 10,000 a diminution of the tone with lessening of the amplitude of contractions is seen (Plate XLII, fig D). The action appears to be a direct one on the muscle. The intestinal muscle behaves in the same way as the uterine muscle.

DISCUSSION

It will be seen from the experimental data given above that Alepol is a drug of comparatively low toxicity and that it has only a feeble action on most of the important systems of the body except the blood. It exerts a marked hæmolytic action on the red blood corpuscle but this effect can be considerably reduced by preparing the solutions in Locke's solution instead of distilled water and adding a small quantity of serum. It is now well known that salts of the fatty acids of the hydncarpus oil have a specific toxic action on the acid-fast bacteria and Alepol too is very highly toxic to the acid-fast bacteria. The successful treatment of leprosy consists in saturating the system with the remedy without producing any untoward effect. One of the greatest drawbacks in doing so is the action of the drug on the red blood corpuscles. It is possible to increase considerably the dose of the drug

if, as has been described before, a combination of serum with Locke's solution is used for giving the injections. When administering the drug by intramuscular injections the irritant nature of the fatty acid salts again is a drawback. This too can be lessened by preparing the solution in Locke's solution. Alepol is less irritant and consequently less painful than other soaps of the fatty acids of the *hydnocarpus* oil and if the solutions are made in the way described the incidence of pain will be considerably smaller. When administering the drug by the intravenous method, the injections must be given as slowly as possible. It is found in animal experiments that symptoms like respiratory distress can be altogether avoided if the rate of injection is very slow. One c.c. per minute is a very slow rate indeed but it is advantageous to inject the solution at this rate.

SUMMARY AND CONCLUSIONS

(i) Alepol is a soap prepared from the lower melting point fatty acids of the *hydnocarpus* oil. It has a fairly low toxicity when compared with other soaps of *hydnocarpus* oil. It inhibits the growth of tubercle bacilli in a concentration of 1 in 200,000 and exerts some toxic action on certain helminths like the tape worms and the microfilariae.

(ii) It has a marked hæmolytic action but this effect can be lessened by dissolving the drug in Locke's solution and adding a small quantity of serum.

(iii) Locally it produces a marked irritation when applied in 5 per cent strengths. Higher concentrations produce a more severe reaction. This action can be reduced by using Locke's solution instead of water.

(iv) Large doses depress the cardio-vascular system. Doses smaller than 10 mg. per kilo do not affect this system to a marked extent.

(v) Respiration is stimulated with large doses of Alepol. Bronchioles are constricted with large doses and dilated with smaller ones.

(vi) Uterine movements are lessened with diminution of tone when the uterus is perfused with strengths higher than 1 in 10,000. With lower concentrations the tone is increased.

(vii) Oral administration leads to gastro-intestinal irritation. Movements of the gastro-intestinal tract are not much affected by intravenous administration of the drug.

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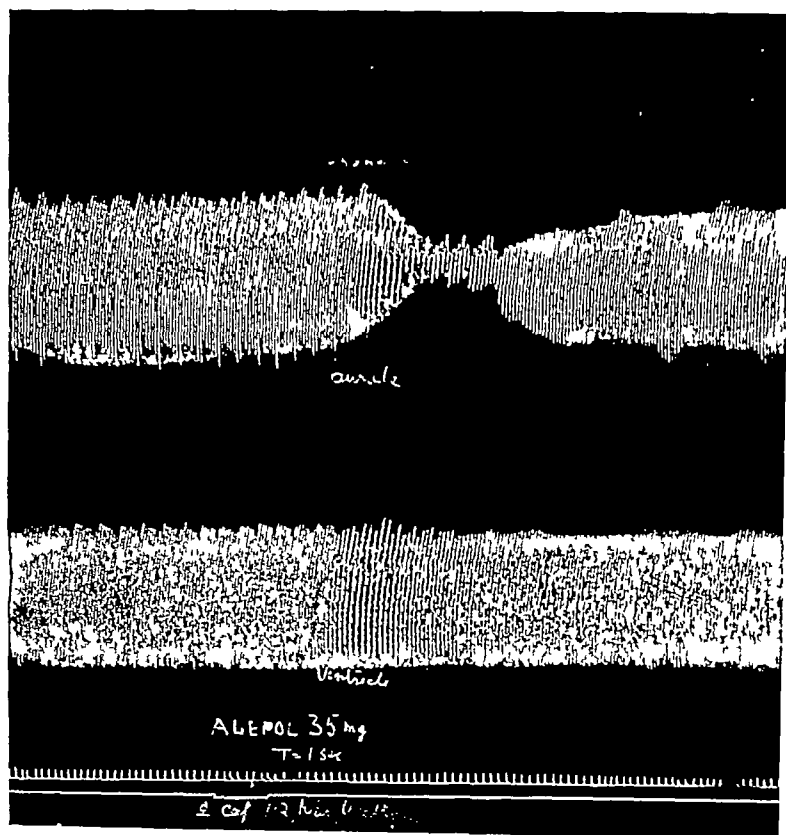


Fig A

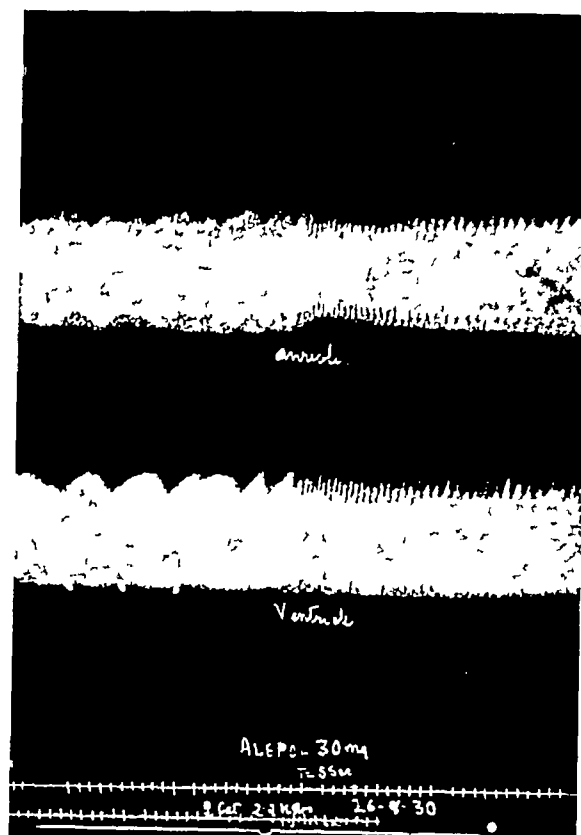


Fig B

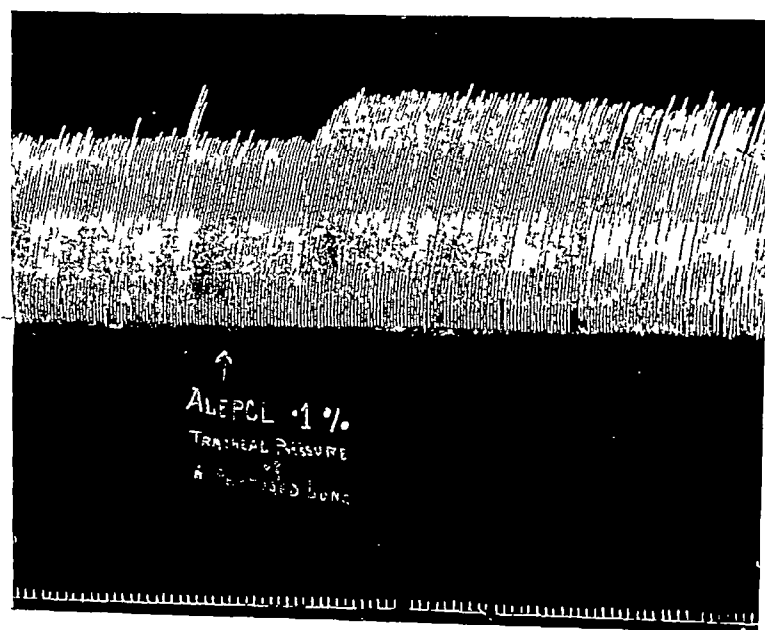


Fig C

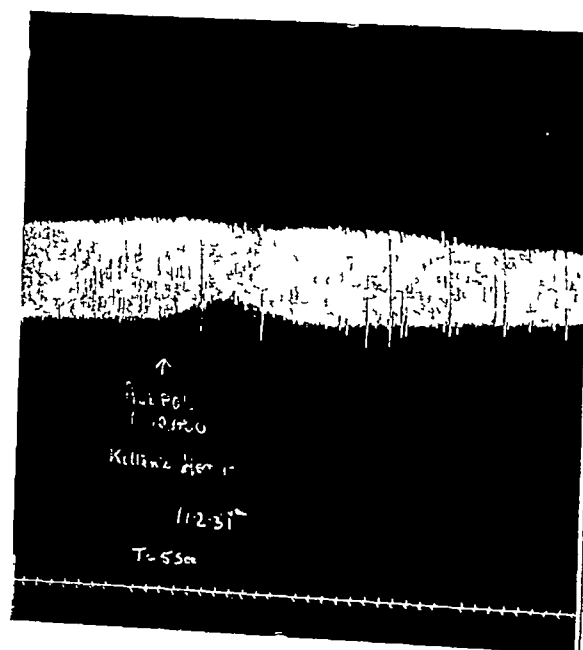


Fig D

- Fig A. Auricular (upper) and Ventricular (lower) tracings of the heart of a cat Alepol (large dose 30 mg p k) given the mark. Note depression of both auricle and ventricle Effect on auricle much more marked
- „ B Similar as Fig A Dose of Alepol is smaller (about 14 mg p k) Note a slight depression of the auricle and ventricle
- „ C Tracheal pressure of perfused lungs with artificial respiration Alepol 1 in 1,000 at arrow mark Note the increase the excursions of the liver showing constriction of bronchioles
- „ D Perfused kitten's heart Alepol 1 in 10,000 at arrow mark Note depression, recovery and again a depression A slight irregularity is also present

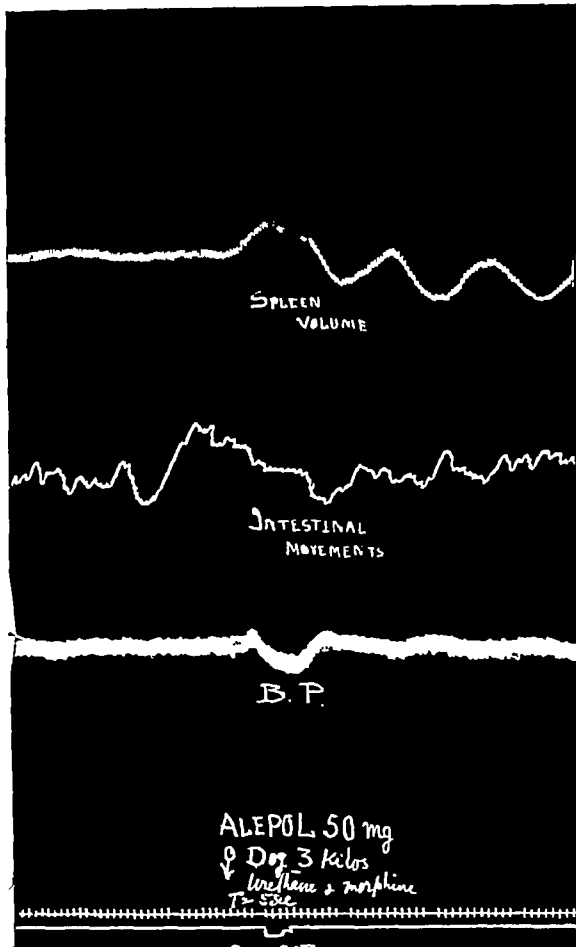


Fig A

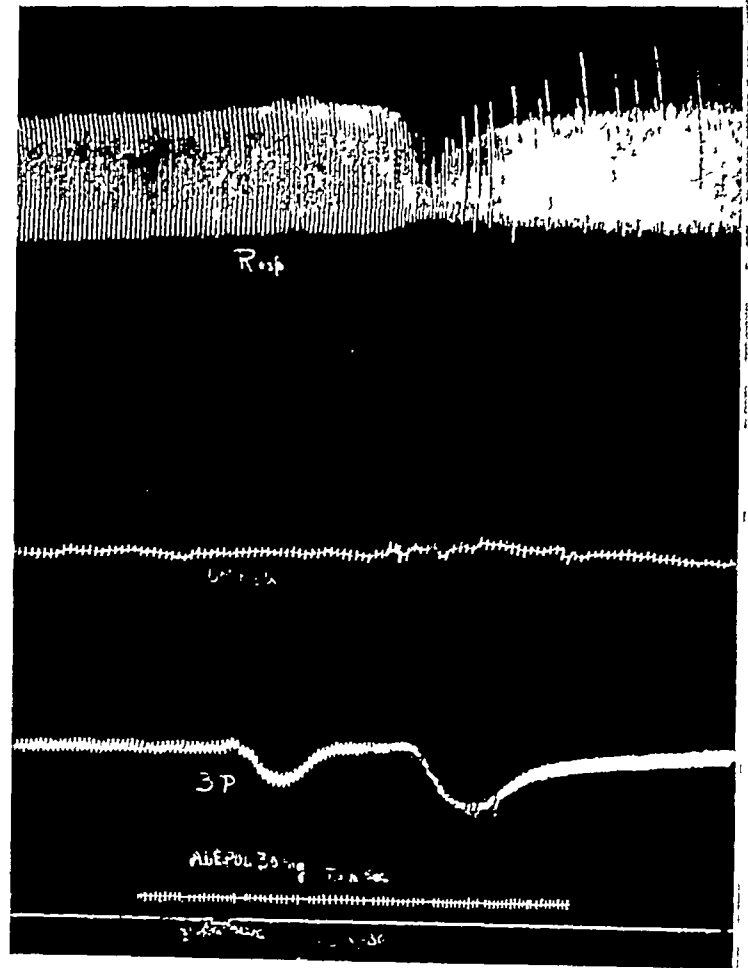


Fig B

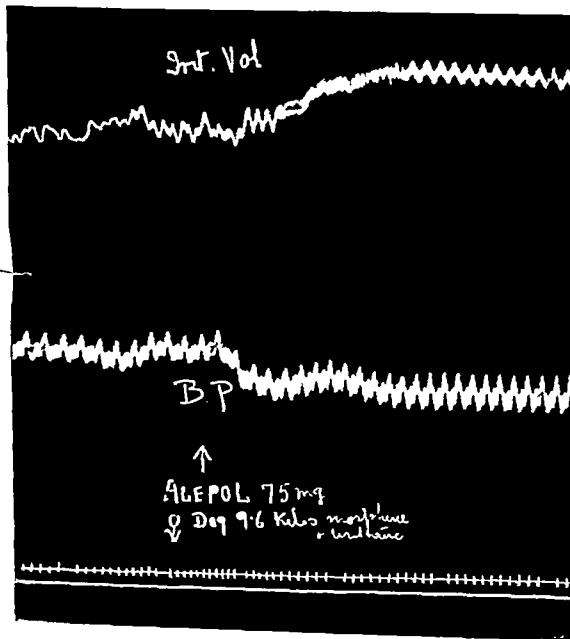


Fig C

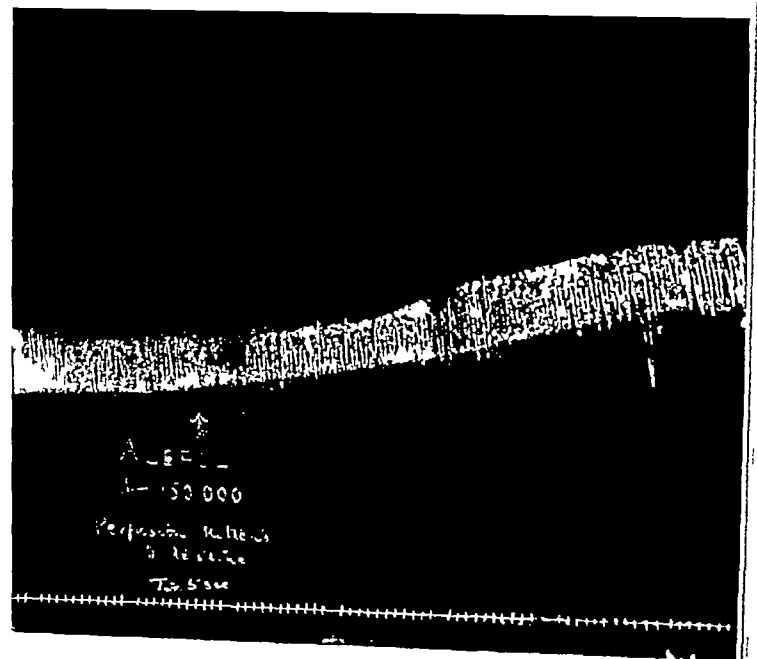


Fig D

- Fig A Shows a slight diminution of the spleen volume and a slight fall of blood pressure after Alepol injection. Movements of the intestines not affected.
- „ B Shows acceleration of respiration and fall in blood pressure after Alepol injection. Movements of uterus (middle tracing) not affected.
- „ C Shows a rise in intestinal volume and fall of blood pressure after Alepol injection.
- „ D Shows an increase in the tone of kitten's intestines in perfusion experiment with 1-150,000 dilution of Alepol.

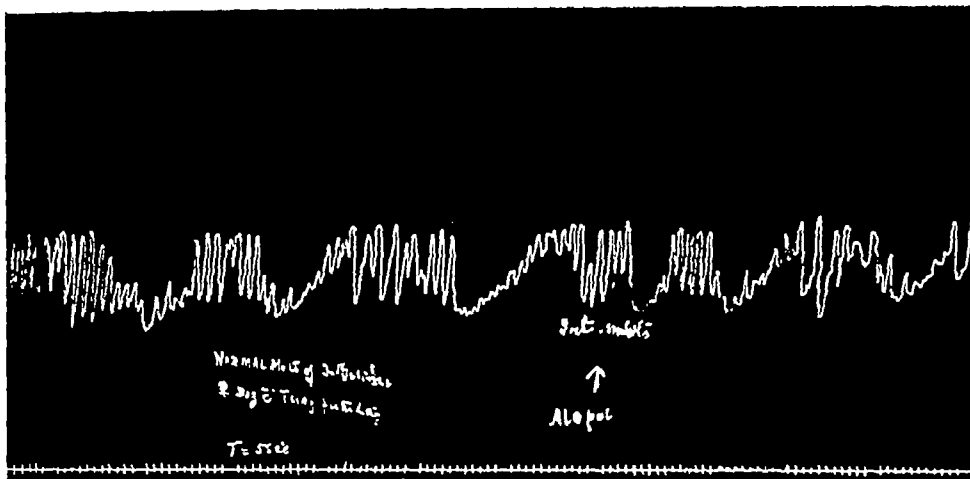


Fig A

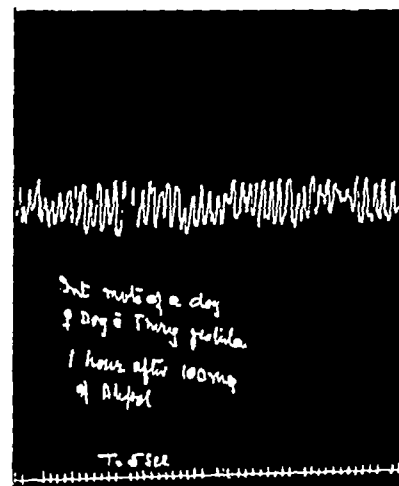


Fig B

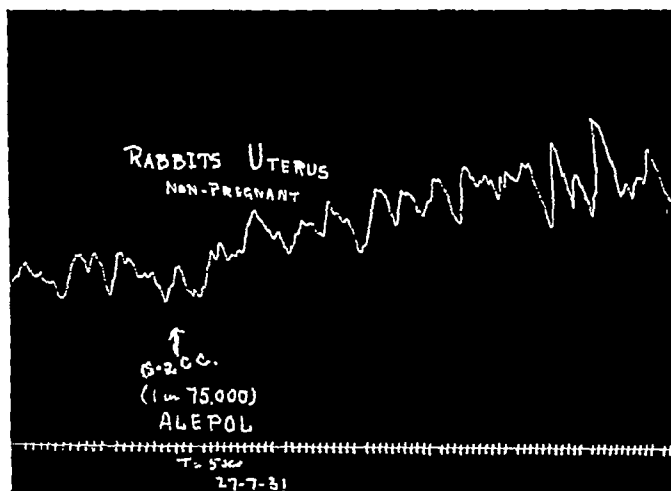


Fig C

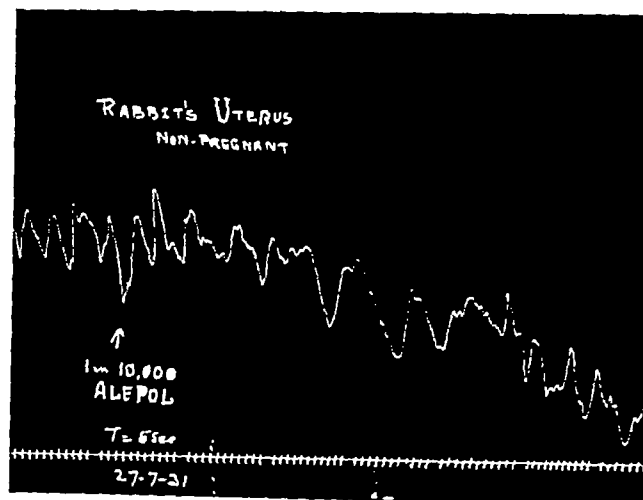


Fig D

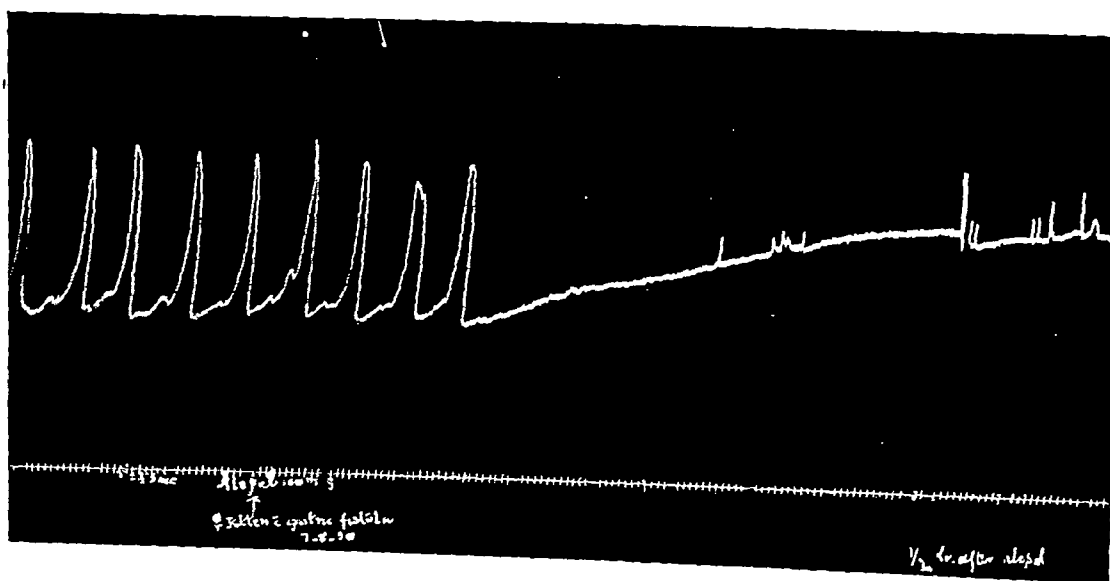


Fig E

- Fig A Shows the movements of the small intestines in a dog with a Thury fistula, Alepol 0.1 g at arrow mark
- " B Same as A taken one hour after Alepol injection Note the slight depression of movements
- Figs C and D Isolated rabbit's uterus Note contraction after Alepol 1-75,000 and relaxation after 1-10,000
- Fig E Stomach movements of a cat with a gastric fistula Alepol 0.1 g at arrow mark Note cessation of movement after about 3 minutes and increase in the tone of the stomach Tracing to the extreme right taken half an hour after Alepol was given The tone remains the same and movements are absent

STUDIES OF THE GENUS *PROTEUS*

Part I.

A CULTURAL AND SEROLOGICAL STUDY OF CERTAIN STRAINS OF THE *PROTEUS* GROUP

Part II.

THE LABORATORY DIAGNOSIS OF TYPHUS FEVER

THE WILSON-WEIL-FELIX REACTION

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[Received for publication, July 20, 1931]

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SUMMARY AND CONCLUSIONS

ACKNOWLEDGMENTS

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Part I.**A CULTURAL AND SEROLOGICAL STUDY OF CERTAIN STRAINS OF THE *PROTEUS* GROUP****INTRODUCTION**

ORGANISMS of the genus *Proteus* have been isolated from various sources. Hauser (1885) isolated them from putrefying meat. Cantu (1911) isolated the *Proteus* from garden soil. Ford (1901) isolated these organisms from various parts of the gastro-intestinal tracts of human cadavers. Waid (1899) secured several strains from the Thames River, and Jordan (1903) from the waters of the upper Mississippi. Horowitz (1916) successfully isolated them from snow-water. Wenner and Rettger (1919) obtained them from stagnant pools, aquaria and street washings, and also from the partly decomposed bodies of rabbits and guinea-pigs.

Considerable confusion has existed until recently as to the pathogenic properties of this group of organisms and the part played by them in human pathology. A common misconception has been that they are non-pathogenic. It is true that they do not probably give rise to any specific disease, but they are frequently associated with and appear to be responsible for a number of inflammatory and suppurative conditions in the human being, especially in connection with infections of the urinary tract, acute gastro-enteritis of children, meat poisoning and Weil's disease or infectious jaundice. It was not, however, until the isolation of certain strains designated X2 and X19 from the urine and blood of some cases of typhus fever by Weil and Felix (1916, 1917) and the discovery of the Weil-Felix reaction that the subject attracted any considerable attention. Some of the earlier references to *Proteus* may be mentioned here —

Krogus (1890) demonstrated its pathogenic power for experimental animals by subcutaneous and intraperitoneal inoculation. Rovsing (1897) regards *Proteus* as a very disagreeable microbe in the urinary tract, chiefly on account of its power

of decomposing urea, and liberating ammonia with resulting irritation of the mucous membrane of the bladder and consequent desquamation of the vesical epithelium. Magath (1928) and Taylor (1928) have found it associated with cystitis and have isolated it from the urine of infected patients. Wesenberg (1898), Silberschmidt (1899), Pfuhl (1900), Schumburg (1902), and Pergola (1910) have mentioned the *Proteus* organisms in connection with outbreaks of meat poisoning caused by consumption of infected sausages. Glucksmann (1899) has described the association of acute gastro-enteritis with the consumption of pork infected with *Proteus*. Baerthlein (1922) described an outbreak of meat poisoning on the Western Front in France in 1918 following the consumption of sausages.

Booker (1889) has charged *Proteus* with the production of summer diarrhoea in children. Metchnikoff (1914) found the *Proteus bacilli* constantly in the stools of children suffering from summer diarrhoea. Bertrand (1914) examined 55 cases of infantile diarrhoea and found *Proteus* organisms in all of them. Tsiklinsky (1917) and Bang (1918) demonstrated the presence of the *Proteus* in cases of infantile diarrhoea.

Flexner (1893) isolated a *Proteus* organism from a case of peritonitis. According to Topley (1929) these organisms are apparently present as secondary invaders in gun-shot wounds, where they favour the development of pathogenic anaerobes. He also mentions them as associated with a variety of conditions, such as volvulus, peritonitis, croupous pneumonia, empyema, gangrene of the lung and septicæmia. Dudgeon, Gardner and Bawtree (1915), Goadby (1916), Distaso (1916), Stewart (1917), Douglas, Fleming and Colebrook (1920) and Weinberg and Otelesco (1921) found *Proteus* strains in gun-shot wounds.

Jensen (1913) considered these organisms responsible for one form of calf dysentery. Wyss (1898) found them in an epidemic disease of fish in Lake Zurich. Kellert (1922) found *Proteus bacilli* in a case of abscess of the brain both during life and at autopsy, and Bischoff and Brekenfeld (1925) reported their presence in a case of meningitis in an infant.

The position of *Proteus bacilli* with regard to putrefaction has been studied by Tissier (1912), who used their proteolytic power as an indicator of their putrefactive properties. He found them less active towards albumin than some of the anaerobic bacteria tested at the same time. Rettger and Newell (1912-13) do not regard *Proteus bacilli* as putrefactive organisms. According to these authors putrefaction is a 'particular process of protein decomposition which is brought about through the agency of bacteria with the evolution of foul-smelling products which are characteristic of ordinary cadaveric decomposition'. Of the products of decomposition they regard mercaptan and the oxy-acids of particular significance, indol, skatol and hydrogen sulphide are less characteristic. Rettger and Newell regarded

putrefaction as an anaerobic process which depends on the growth of obligatory anaerobes. They say that *Proteus bacilli* under anaerobic conditions do not digest proteins, and therefore can not be regarded as capable of causing true putrefaction. They are, however, according to Topley (1929), frequently associated with anaerobes in putrefying organic material and by using up oxygen render the conditions favourable for the development of these organisms.

Jaeger (1892) ascribed an ætiological rôle in Weil's disease to an organism classified by him as *Bacillus proteus fluorescens* and which resembled *Proteus vulgaris* except in the matter of greenish pigment. This organism was isolated from the urine, blood and organs of patients dead of the disease. Although not now generally regarded as the causal agent, agglutination of *Proteus fluorescens* and *Proteus vulgaris* by the sera of patients having Weil's disease has been reported by several authors, namely, Ludke (1904), Bruning (1904) and Abeles (1906-07).

Lastly the organisms of this group have been mentioned in connection with typhus fever. The relationship of *Proteus bacilli* to the virus of typhus fever was brought into prominence by the investigations of Weil and Felix (1916, 1917). These authors demonstrated that of the various organisms isolated from typhus fever patients one strain was capable of giving a specific agglutination reaction with sera of patients with typhus fever. It was not agglutinable with normal human sera or sera of persons suffering from other diseases. Many members of this strain were isolated, of which the most important were X19 and X2. The exact relationship between the typhus virus and these strains has not been established, although it is not improbable that there is a near antigenic relation between these organisms and the typhus virus.

The above-mentioned facts indicate that the bacteriology of the *Proteus* group of organisms is in a somewhat chaotic condition, and therefore further study of the subject is desirable.

SOURCE OF THE CULTURES

The designation and source of each of the cultures studied is shown in Table I. Altogether 36 strains were examined.

Before detailed examination each strain was plated out on nutrient agar. Streak plates were made and incubated for 18 hours at 37°C. The isolation was carried out by inoculation with a straight needle from the outer border of the swarming edge of a colony, in the case of swarming strains, and from a single well isolated colony in the case of non-swarming strains into plain broth. The broth cultures after 24 hours' incubation were used for gelatine stabs and other tests.

TABLE I.

List of strains studied

Serial number	Designation	Source	REMARKS
1	X2 O	Dr K F Maxey, Virginia University, Virginia	Originally obtained from Dr A Felix
2	X19 O	Do	Do
3	X19 H	Do	Do
4	X19 (N Y)	U S Quarantine Station New York	The strain carried by the New York State Health Department
5	X19 (Feirer)	Dr Wilham A Feirer	
6	U2	Dr K F Maxey	<i>B agglutinabilis</i> Isolated in 1909 by W J Wilson from the feces of a typhoid fever patient
7	Kingsbury O	Do	Originally obtained from the Director, Institute of Medical Research, Kuala Lumpur, Federated Malay States
8	Kingsbury H	Do	Do
9	526	Do	No 221 of the American Type Collection
10	<i>Proteus</i> I	Dr L B Lange, Johns Hopkins School of Hygiene	Originally obtained from Dr Kraus, Vienna
11	<i>Proteus</i> II	Do	Obtained from the Bacteriological Laboratory, Johns Hopkins Medical School
12	<i>Proteus</i> III	Do	Isolated in 1919 from an infant's stools
13	<i>Proteus</i> V	Do	Originally obtained from Dr Leo F Rettger Sheffield Bacteriological Laboratory, Yale University
14	<i>Proteus mirabilis</i>	Do	Do
15	<i>Proteus</i> 6A	Dr W W Ford, Johns Hopkins School of Hygiene	Isolated from war wounds on the Western Front in France during the World War
16	<i>Proteus</i> 752A	Do	Do
17	<i>Proteus</i> 78071A	Do	Do
18	<i>Proteus</i> 754A	Do	Do

TABLE I—*concl'd*

Serial number	Designation	Source	REMARKS
19	<i>Proteus</i> 91466A	Dr W W Ford, Johns Hopkins School of Hygiene	Isolated from war wounds on the Western Front in France during the World War
20	<i>Proteus</i> 692A	Do	Do
21	<i>Proteus</i> B7A2	Do	Do
22	<i>Proteus</i> 124S C	Do	Isolated from the intestinal tract of a calf
23	<i>Proteus</i> 115	Do	Do
24	<i>Proteus</i> 115A	Do	Do
25	<i>Proteus</i> 124S W	Do	Do
26	<i>Proteus</i> R I	Dr C P Eliot	Isolated from the intestinal tract of a rat
27	Strain A	Dr C A Perry, State Health Department, Maryland	Originally obtained from the Hygiene Laboratory, Washington, D X19 No 568
28	Strain B	Do	Hygiene Laboratory, Strain H X19
29	Strain C	Do	Parke Davis & Co, Strain X19, No 02165
30	Strain D	Do	Obtained from Dr H Pinkerton, Department of Pathology, Harvard University. Brought from Mexico by Dr Castaneda. Believed to have come originally from Europe. <i>Proteus vulgaris</i>
31	Strain E	Do	
32	Strain F	Do	Strain X2 (F C), Pasteur Institute, Paris
33	Strain G	Do	Strain X19 (Sarie), Pasteur Institute, Paris
34	Strain H	Do	Strain X19 (Job Metz), Pasteur Institute, Paris
35	Strain I	Do	Strain O X19, Pasteur Institute, Paris
36	Strain J	Do	Strain X19 (F C) Pasteur Institute, Paris

MORPHOLOGICAL CHARACTERS OF THE *Proteus* GROUP*Growth on solid media*

This group of organisms was first fully described by Hauser in 1885. He isolated them from putrefying meat and created the genus *Proteus*. He named it *Proteus* in allusion to the ever-changing appearance of the 'old man of the sea' as described in the Homeric poems, thus stressing the highly pleomorphic character of this group of organisms. Hauser subdivided the genus into three species, which he named *Proteus vulgaris*, *Proteus mirabilis* and *Proteus zenkeri*. *Proteus vulgaris* liquefied gelatine rapidly, *Proteus mirabilis* more slowly, whilst *Proteus zenkeri* did not liquefy gelatine at all. Later, however, Hauser (1892) modified his statement and regarded the two last mentioned forms as variations of the same species, namely *Proteus vulgaris*. According to him the members of this group were characterized by being highly motile rods, capable of liquefying gelatine either immediately or after several transfers, and capable of spreading on gelatine.

The phenomenon of swarming or spreading was further described by Cantu in 1911. He found that if an organism of the *Proteus* group was inoculated into the water of condensation of an agar slope, the growth which took place rapidly invaded the whole surface of the slope, producing a uniform layer sometimes hardly distinguishable from the medium. Choukevitch (1911) also made use of this property for the isolation of organisms of the *Proteus* group from a mixture. The process has been more fully described recently by Moltke (1927). He states that this is the only criterion besides gelatine liquefaction on which the diagnosis of *Proteus* according to Hauser's description can be based. Moltke regards swarming as a 'progressive surface spreading by the microbes from the edge of the colony, without the colony taking any apparent part in the process. There is—particularly in young cultures—a sharp line between the colony and the swarming part. In this feature *Proteus bacilli* differ distinctly from the other spreading bacteria (*Bacillus subtilis*, *Bacillus zopfii*, *Bacillus pyocyaneus*), in which the surface spreading is done by the colony itself. By its swarming *Bacillus proteus* covers, at a surprising speed, the substrate with a greyish moist film—another contrast to the bacteria mentioned above, whose surface membrane is dry and wrinkled.'

Weil and Fehx (1917) during their morphological investigations of the *Proteus* organisms have described two kinds of colonies, the swarming and the non-swarming. In an X19 strain they found both kinds of colonies—'H', i.e., mit Hauch or swarming type, and the 'O', i.e., ohne Hauch or non-swarming type. They have demonstrated serological differences between the two types.

In this investigation all the strains were studied for their behaviour with regard to swarming. This was carried out by touching the centre of an agar plate with a needle dipped into a 24-hour broth culture of the organism. The plate was incubated at 37°C, and examined 6 to 8 hours later and again after 24 hours. It

was noticed that at first one or two colonies developed which later spread over the whole surface of the plate as a thin ground glass-like pellicle hardly distinguishable in some cases from the medium itself. In Plate XLIV, fig. 1, a swarming colony on agar has been shown. This phenomenon was also studied on 12 per cent gelatine plates, on which the process was found to be still more rapid. The gelatine, however, became rapidly liquefied and, therefore, it could not be followed up so well on this medium as on agar. Under low powers of the microscope the colonies on this medium appeared more or less thick and opaque in the centre with peculiar fungus-like prolongations protruding from the margins.

In addition, each strain was inoculated into the water of condensation of an agar slope by means of a needle dipped into a broth culture of the organism. Twenty-four hours later in the case of a swarming strain it was found to have climbed to the top of the slope, and at the same time to have spread over the entire surface. As described by Taylor (1928) the film, whether formed by spreading or climbing, had a shiny greasy appearance and usually showed small variations in thickness in the form of ripples. These were visible even when the growth of the organism was so thin and translucent as to be almost invisible (Plate XLIV, fig. 2). With the increase of age the growth on agar usually assumed a greyish white butyrous appearance.

During the process of swarming it was an almost invariable rule, in the majority of cases, to find the long filamentous forms, whereas after 48 hours or so when the swarming had more or less reached its limit in most cases, the long forms were replaced by short rods or even coccoid forms.

All the strains examined were found to be swimmers or spreaders with the exception of X2 O, X19 O, Kingsbury O and Strain D. These strains gave rise to small raised colonies—the 'O' colonies of Weil and Felix—resembling those of *Bacillus coli*. It may be mentioned here that early in the investigation the strain X19 O was found to have suddenly reverted to the 'H' form, i.e. to have developed swarming tendencies. Attempts to isolate the 'O' form by a process of repeated plating proved unsuccessful. Fortunately, a transfer from the original culture was still available and was found to have retained its 'O' form.

Strain U2 also did not swarm, but this organism, which was originally isolated by Wilson of Belfast (1909), is not a member of the *Proteus* group. It is non-motile, produces acid and gas in lactose and was described by Wilson as 'a variant of the *Bacillus coli*'. It has been included in this study because it is stated by Wilson to be agglutinable with typhus sera.

Strain I, although stated to be an X19 O strain, was found to possess swarming properties.

Growth in liquid media

All the strains were inoculated into nutrient broth and peptone water and incubated at 37°C. In these media uniform turbidity was visible in 24 hours with

PLATE XLIV

Colonies of Proteus X19
after 24 hours on nutrient agar



Fig. 1 —An 'H' or swarming colony X400



Fig. 2 —Film of swarming colonies showing ripples.

the presence of long, filamentous and sometimes vibrio-like forms. After 48 hours a greyish white deposit tended to appear at the bottom of the tube, and the majority of the organisms changed into the short rod form. In some cases, all the forms, filamentous, long, medium, short and even coccoid were found in the same medium at the same time. Older cultures usually developed a thin fragile pellicle.

Staining properties of the Proteus group

Various authors, specially the earlier ones, have expressed different opinions as to the reactions of *Proteus bacilli* to Gram staining. Some have considered it as Gram negative, others as Gram positive. This confusion has arisen chiefly because the *Bacillus zopfii*, which is Gram positive, has been regarded as a member of the *Proteus* group.

Krogus (1890) and Schmitzler (1890) found all their strains Gram negative. Meyerhof (1898), Wesenberg (1898) and Silberschmidt (1899) also report the same. Kheneberger (1908) studied 24 strains, of which 18 were Gram negative. Cantu (1911) examined 180 strains and found them all Gram negative. Neumann (1912) says that *Proteus vulgaris* is sometimes Gram positive and sometimes Gram negative. Heim (1913) regards *Proteus vulgaris* as usually Gram negative as compared with *Bacillus zopfii* which is always Gram positive. Berdnikov (1914) examined 6 strains, 2 of which he regarded as members of the *Proteus* group. These were Gram negative, whilst the other 4, which he considered as *Bacillus zopfii* were Gram positive. Berthelot (1914) studied a large number of strains, all of which were Gram negative. Horowitz (1916) examined 24 strains which gave variable reactions, but the majority of them were Gram negative. Weil and Felix (1916) considered their X strains as all Gram negative. Bengtson (1919) studied 33 strains of *Proteus vulgaris* and found all of them Gram negative. Wenner and Rettger (1919) report all their 73 strains as Gram negative. Douglas, Fleming and Colebrook (1920), who isolated 40 strains from infected wounds, found them all Gram negative. Moltke (1927) after an exhaustive study of 194 strains of *Proteus* found them all Gram negative. Taylor (1928) studied 53 strains, all of which he regarded as Gram negative.

Ford (1927) and Topley (1929) consider *Proteus vulgaris* to be Gram negative. In 'A System of Bacteriology in Relation to Medicine', Vol. IV, issued by the Medical Research Council, Wilson (1929) states that the *Proteus vulgaris* 'stains readily with the ordinary basic coal tar dyes and is Gram negative'.

The confusion regarding the staining properties of *Proteus* has arisen because Kurth (1883) obtained an organism from the intestinal tract of fowls which he designated as *Bacillus zopfii*, and Hauser (1885) described an organism which he named *Proteus zenkeri*, which in later comparative studies have been shown to be identical by Wenner and Rettger (1919) and Ford (1927). These organisms were Gram positive, did not liquefy gelatine, failed to produce indol and did not

attack carbohydrates. Consequently Beigev (1926) created a new genus—*Kuntzia*—in which these two species were designated as *Kuntzia zopfii* and *Kuntzia zenkeri*, although it is difficult to see why the separation is maintained in view of the identity of the organisms.

In the present study all the strains were found to decolorize with Gram's method. They also stained evenly with 5 per cent methylene blue and 1 in 10 carbol fuchsin. No irregular or meta-chromatic staining was observed.

Microscopic appearance and motility

Microscopic examination of stained preparations showed all the strains to be similar in morphology. They were non-sporebearing bacilli with rounded ends. The variability in length was dependent on the conditions of growth and the age of the culture. The usual size was 1 to 3μ by $0.3-0.5\mu$. In some cultures, however, forms 6 to 8μ in length or even much longer—20 to 30μ —were found.

Hauser (1885) described *Proteus* as a motile organism. Since then various authors have laid emphasis on this character of the group, the 'O' forms of Weil and Felix being the only exceptions.

In this study each strain was examined for motility by growing it in plain broth for 24 hours. All the *Proteus* strains were thus found to be actively motile with the exception of X2 O, X19 O, Kingsbury O and Strain D which were non-motile. It was observed that generally there was a direct relationship between motility and the swarming capacity of a strain. All actively motile strains were also vigorous swimmers, while the non-motile strains were found to be devoid of this property. The results of certain comparative culture studies and observations on motility are shown in Table II —

TABLE II

Serial number	Designation	Motility	Swarming	Gelatin liquefaction	Serum liquefaction	H ₂ S formation	Urea decomposition	Indol production	Maltose fermentation
1	X2 O	—	—	+	—	+	+	+	+
2	X19 O	—	—	+	—	+	+	+	+
3	X19 H	+	+	+	—	+	+	+	+
4	X19 (N Y)	+	+	+	—	+	+	+	+
5	X19 (Foner)	+	+	+	—	+	+	+	+
6	U2	—	—	—	—	+	—	—	+
7	Kingsbury O	—	—	—	—	+	+	—	—
8	Kingsbury H	+	+	—	—	+	+	—	—
9	526	+	+	+	+	+	+	—	—
10	<i>Proteus</i> I	+	+	+	—	+	+	+	+

TABLE II—*concl'd*

Serial number	Designation	Motility	Swarming	Gelatin liquefaction	Serum liquefaction	H ₂ S formation	Urea decomposition	Indol production	Maltose fermentation
11	<i>Proteus</i> II	+	+	+	—	+	+	+	+
12	<i>Proteus</i> III	+	+	+	—	+	+	+	+
13	<i>Proteus</i> V	+	+	+	—	+	+	+	+
14	<i>Proteus mirabilis</i>	+	+	+	—	+	+	—	—
15	<i>Proteus</i> 6A	+	+	+	+	+	+	+	+
16	<i>Proteus</i> 752A	+	+	+	+	+	+	—	—
17	<i>Proteus</i> 78071A	+	+	+	+	+	+	—	—
18	<i>Proteus</i> 751A	+	+	+	—	+	+	—	—
19	<i>Proteus</i> 91466A	+	+	+	—	+	+	+	+
20	<i>Proteus</i> 692A	+	+	+	—	+	+	+	+
21	<i>Proteus</i> B7A2	+	+	+	—	+	+	+	—
22	<i>Proteus</i> 124S C	+	+	+	+	+	+	—	—
23	<i>Proteus</i> 115	+	+	+	—	+	+	+	+
24	<i>Proteus</i> 115A	+	+	+	—	+	+	—	—
25	<i>Proteus</i> 124S W	+	+	+	—	+	+	—	—
26	<i>Proteus</i> R I	+	+	+	—	+	+	+	+
27	Strain A	+	+	+	—	+	+	+	+
28	Strain B	+	+	+	—	+	+	+	+
29	Strain C	+	+	+	—	+	+	+	+
30	Strain D	—	—	—	—	+	+	+	+
31	Strain E	+	+	+	—	+	+	+	+
32	Strain F	+	+	+	—	+	+	+	+
33	Strain G	+	+	+	—	+	+	+	+
34	Strain H	+	+	+	—	+	+	+	+
35	Strain I	+	+	+	—	+	+	+	+
36	Strain J	+	+	+	—	+	+	+	+

CULTURAL CHARACTERS OF THE *Proteus* GROUP*Fermentation reactions*

Theobald Smith first studied the fermentation reactions of *Proteus* in 1893. He found that it fermented glucose and saccharose with the formation of acid and gas, but not lactose. He (1894) emphasized the importance of this reaction in the diagnosis of *Proteus*, i.e., the fermentation of glucose and saccharose and the absence of fermentation of lactose as being characteristic.

Jordan (1903) stated that *Proteus* always ferments dextrose and saccharose but never lactose.

Berthelot (1914) studied a number of *Proteus* strains. He found that mannite and lactose were never fermented, while maltose was rapidly fermented by some strains and not at all by others.

Besson, Ranque and Senez (1918) studied the fermentation reactions of one *Proteus* organism. This was found to ferment glycerine, glucose and levulose, but not mannite, dulcitol, saccharose and lactose.

Jotten (1919) found that the X strains fermented maltose and saccharose. Bengtson (1919) studied the fermentation reactions of a number of strains. She found that all her strains fermented levulose, galactose, xylose, glycerine, dextrose and saccharose, and some were shown to have produced acid in mannite. Some fermented maltose also. All maltose and saccharose fermenting strains formed indol as well.

Wenner and Rettger (1919) studied the reactions of 73 strains on a number of carbohydrates. All strains fermented glycerine, dextrose, galactose, levulose and saccharose. Twenty-five strains fermented maltose. Lactose, mannite and dextrin were not fermented by any. They regarded the fermentation of maltose as a basis on which sub-grouping might be attempted. For the species fermenting this carbohydrate they suggested the name of *Proteus vulgans*, and for the species failing to attack it the name *Proteus mirabilis*.

Douglas Fleming and Colebrook (1920) emphasized the difficulties connected with the fermentation tests of *Proteus bacilli*, since these microbes destroy most of the indicators. They are, however, inclined to the view that members of the *Proteus* group do not ferment lactose and mannite.

Moltke (1927) examined 171 strains on a large number of sugars and polyhydric alcohols. Amongst important properties he mentioned their power to produce acid and gas in dextrose, galactose, saccharose, xylose, trehalose and glycerine, their failure to ferment lactose, mannose, dextrin, mannite or any of the polyhydric alcohols. Of the 171 strains tested 37 fermented maltose and the rest did not. The maltose positive strains were found invariably to produce indol, whereas the maltose negative strains failed to do so.

Taylor (1928) studied 53 strains derived from various sources. Of these only 3 fermented maltose. None fermented lactose, mannite or dulcitol. All fermented dextrose and saccharose.

Topley (1929) considers that the classification of the *Proteus* group can be best made on the basis of maltose fermentation.

In this investigation the culture medium employed consisted of nutrient broth containing 1 per cent of the particular carbohydrate required. The medium was tubed in Durham's fermentation tubes and then exposed to streaming steam in the Arnold steam sterilizer for 15 minutes on three consecutive days.

Altogether 36 strains were studied on the following —

Lactose, dextrose, saccharose, maltose, levulose, galactose, arabinose, xylose, glycerine, mannite, dulcitol, sorbitol, salicin, dextrin, starch, milk and litmus milk.

The media were inoculated from broth cultures of the organisms, and kept at a temperature of 37°C for a period of three weeks. The results were noted every

24 hours. Andrade's indicator was employed throughout. The results of the fermentation reactions are shown in Table III. 'A' indicates acid and 'G' gas formation, '+' under A or G means acid or gas formation, '—' stands for negative, 'R' stands for reduction and, 'D' for digestion of litmus milk.

It will be noted that all strains ferment dextrose and glycerine. None ferments lactose, except U2, which as already stated is not a member of the *Proteus* group.

All strains also ferment saccharose. The Kingsbury strains are stated by Fletcher and Lessler (1925, 1926) not to ferment saccharose or maltose. In my hands, however, both the strains—Kingsbury O and Kingsbury H—have repeatedly fermented saccharose in 48 hours but not maltose.

None of the strains with the exception of U2—a member of the *Bacillus coli* group—ferments mannite, although Bengtson (1919) considers that *Proteus vulgaris* frequently does ferment mannite. After a survey of the question she concludes 'It seems probable that faecal strains often ferment maltose, saccharose and mannite when freshly isolated, and later lose the power to a greater or less extent and sometimes completely.'

As regards maltose 25 strains ferment this carbohydrate including U2, and 11 strains do not. The X2 and all the X19 strains belong to the former group.*

Salicin is fermented by 18 strains only, including the X strains. The Kingsbury strains, although stated to be descendants of an X19 strain, again behave differently. They fail to ferment salicin. It was also noticed that a number of maltose positive strains ferment salicin rapidly—within 48 hours.

Levulose, galactose, xylose, arabinose and trehalose are fermented by all strains.

Dextrin, starch, inulin, dulcitol and sorbitol are not acted upon by any strain. The only exception is U2 which forms acid in starch and acid and gas in dextrin and dulcitol.

In litmus milk a series of characteristic changes were observed in the case of all *Proteus* strains. Rapid and vigorous growth takes place in this medium with a highly unpleasant pungent odour. After a transient acidity in some cases the milk is rendered alkaline, and in from 48 to 96 hours reduction of the litmus takes place, when the liquid assumes a brownish tint. This is followed by digestion of the casein, the whole process occupying about a fortnight.

The properties of the *Proteus* group are admirably summed up by Besson and Ehringer (1923) as follows —

'Bacilli with rounded ends, very variable in length, no spores, actively motile, numerous peritrichial cilia, Gram negative. Facultative aerobe, giving on gelatine, colonies with tortuous radiating projections creeping out from the parent, on

* Sometimes in about 48 hours a tiny bubble of gas was noticed in the lactose tubes of the X19 strains, but the process did not proceed any further and no acid was ever produced.

agar a culture spreading and climbing Coagulating milk without acidification, then redissolving the clot Not fermenting lactose, mannite or dulcitol, fermenting glucose, levulose, glycerine and often maltose and saccharose with formation of gas Reducing neutral red Producing sulphuretted hydrogen Producing usually indol in peptone water They further say that organisms possessing the above characteristics may show slight differences in proteolytic action, indol production and action on sugars which do not justify the creation of different species, some may be called different types or varieties

The gas formula of the *Proteus* group was determined by growing several strains for 48 hours at 37°C in Smith's fermentation tubes The average formula thus worked out for a number of strains was $H/CO_2 = 3.4/1$

Other reactions

Voges-Proskauer reaction—Very few authors have referred to the Voges-Proskauer reaction as a means of identification for organisms of the *Proteus* group Archibald (1913) found the reaction positive in the case of an organism isolated by him from a case of choleraic diarrhoea Kligler (1914) studied 6 laboratory strains and found that all gave a negative reaction Bengtson (1919) examined a large number of *Proteus vulgaris* strains, none of which gave a positive result

In this study each strain was grown in glucose phosphate medium and the test carried out after 5 days' incubation at 37°C All strains gave negative results

Positive *methylene blue reductase*, *methyl red* and *catalase tests* were given by all strains Likewise *nitrates* were reduced to *nitrites* and *ammonia* was produced by all

Hæmolysis—Wenner and Rettger (1919) tested a number of *Proteus* strains and found that these organisms were unable to hæmolyse red blood cells Bach (1921) showed that a hæmotoxin was present in young broth cultures of *Bacillus proteus* Maximum hæmolysis of sheep and rabbit cells occurred with cultures 5 to 6 hours old Weinberg and Otelesco (1921) studied 8 strains and found that all produced an hæmolysin Taylor (1928) examined 53 strains and reported that all produced an hæmolysin for human red cells when grown in peptone water containing 0.85 per cent sodium chloride His results, however, were negative on blood agar plates

In this study blood agar plates containing rabbit's whole blood were used Tubes containing 10 c.c. of nutrient agar were melted and cooled to 45°C, 0.5 c.c. of rabbit blood obtained by cardiac puncture was added and evenly mixed with the agar The medium was inoculated with a loopful of a 6 hours' broth culture and poured into Petri dishes 4 inches in diameter The plates were incubated at 37°C for 24 hours They were then examined and the appearance noted They were re-examined after another 24 hours' incubation at 37°C, and finally after a further 24 hours in the ice-chest By this method well marked hæmolytic action

was observed in each instance, the hæmolysis being of the *Beta* type. The colonies were surrounded by sharply defined, clear, colourless zones of hæmolysis. Under the microscope no corpuscles could usually be seen within the zones.

Odour—A peculiar unpleasant penetrating odour together with a smell of sulphuretted hydrogen was usually perceptible in the case of all strains except U2 after growth in media containing peptone. The odour was also specially well marked in litmus milk.

TABLE III

Fermentation reactions

Serial number	Designation	Lactose	Dextrose	Saccharose	Maltose	Levulose	Galactose	Ambinose	Trehalose	Xylose
		A G	A G	A G	A G	A G	A G	A G	A G	A G
1	X2 O	--	++	++	++	++	++	++	++	++
2	X19 O	--	++	++	++	++	++	++	++	++
3	X19 H	--	++	++	++	++	++	++	++	++
4	X19 (N Y)	--	++	++	++	++	++	++	++	++
5	X19 (Feirer)	--	++	++	++	++	++	++	++	++
6	U2	++	++	++	++	++	++	++	++	++
7	Kingsbury O	--	++	++	--	++	++	++	++	++
8	Kingsbury H	--	++	++	--	++	++	++	++	++
9	526	--	++	++	--	++	++	++	++	++
10	<i>Proteus</i> I	--	++	++	++	++	++	++	++	++
11	<i>Proteus</i> II	--	++	++	++	++	++	++	++	++

A = Acid, G = Gas

TABLE III—contd

Serial number	Designation	Lactose	Dextrose	Saccharose	Maltose	Levulose	Galactose	Arabinose	Trehalose	Xylose
		A G	A G	A G	A G	A G	A G	A G	A G	A G
12	<i>Proteus</i> III	--	++	++	++	++	++	++	++	++
13	<i>Proteus</i> V	--	++	++	++	++	++	++	++	++
14	<i>Proteus mirabilis</i>	--	++	++	--	++	++	++	++	++
15	<i>Proteus</i> 6A	--	++	++	++	++	++	++	++	++
16	<i>Proteus</i> 752A	--	++	++	--	++	++	++	++	++
17	<i>Proteus</i> 78071A	--	++	++	--	++	++	++	++	++
18	<i>Proteus</i> 754A	--	++	++	--	++	++	++	++	++
19	<i>Proteus</i> 91466A	--	++	++	++	++	++	++	++	++
20	<i>Proteus</i> 692A	--	++	++	++	++	++	++	++	++
21	<i>Proteus</i> B7A2	--	++	++	--	++	++	++	++	++
22	<i>Proteus</i> 124S C	--	++	++	--	++	++	++	++	++
23	<i>Proteus</i> 115	--	++	++	++	++	++	++	++	++
24	<i>Proteus</i> 115A	--	++	++	--	++	++	++	++	++
25	<i>Proteus</i> 124S W	--	++	++	--	++	++	++	++	++
26	<i>Proteus</i> R I	--	++	++	++	++	++	++	++	++
27	Strain A	--	++	++	++	++	++	++	++	++
28	Strain B ^{''}	--	++	++	++	++	++	++	++	++
29	Strain C	--	++	++	++	++	++	++	++	++
30	Strain D	--	++	++	++	++	++	++	++	++
31	Strain E	--	++	++	++	++	++	++	++	++
32	Strain F	--	++	++	++	++	++	++	++	++
33	Strain G	--	++	++	++	++	++	++	++	++
34	Strain H	--	++	++	++	++	++	++	++	++
35	Strain I	--	++	++	++	++	++	++	++	++
36	Strain J	--	++	++	++	++	++	++	++	++

A = Acid, G = Gas

TABLE III—*contd**Fermentation reactions*

Serial number	Designation	Glycerine	Mannite	Dulcite	Sorbite	Salicin	Dextrin	Starch	Inulin	Litmus milk
		A G	A G	A G	A G	A G	A G	A G	A G	A G
1	X2 O	++	--	--	--	++	--	--	--	++
2	X19 O	++	--	--	--	++	--	--	--	++
3	X19 H	++	--	--	--	++	--	--	--	++
4	X19 (N Y)	++	--	--	--	++	--	--	--	++
5	X19 (Feirer)	++	--	--	--	++	--	--	--	++
6	U2	++	++	++	--	++	++	+-	--	++
7	Kingsbury O	++	--	--	--	--	--	--	--	++
8	Kingsbury H	++	--	--	--	--	--	--	--	++
9	526	++	--	--	--	--	--	--	--	++
10	<i>Proteus</i> I	++	--	--	--	--	--	--	--	++
11	<i>Proteus</i> II	++	--	--	--	--	--	--	--	++
12	<i>Proteus</i> III	++	--	--	--	--	--	--	--	++
13	<i>Proteus</i> V	++	--	--	--	--	--	--	--	++
14	<i>Proteus mirabilis</i>	++	--	--	--	--	--	--	--	++
15	<i>Proteus</i> 6A	++	--	--	--	++	--	--	--	++
16	<i>Proteus</i> 752A	++	--	--	--	++	--	--	--	++
17	<i>Proteus</i> 78071A	++	--	--	--	--	--	--	--	++
18	<i>Proteus</i> 754A	++	--	--	--	--	--	--	--	++
19	<i>Proteus</i> 91466A	++	--	--	--	--	--	--	--	++
20	<i>Proteus</i> 692A	++	--	--	--	++	--	--	--	++
21	<i>Proteus</i> B7A2	++	--	--	--	--	--	--	--	++
22	<i>Proteus</i> 124S C	++	--	--	--	--	--	--	--	++
23	<i>Proteus</i> 115	++	--	--	--	--	--	--	--	++

A = Acid, G = Gas

TABLE III—*concl'd*

Serial number	Designation	Glycerine	Mannite	Dulcite	Sorbite	Schlen	Dextrin	Starch	Inulin	Lactus milk
		A G	A G	A G	A G	A G	A G	A G	A G	A G
24	<i>Proteus</i> 115A	++	--	--	--	--	--	--	--	++
25	<i>Proteus</i> 124S W	++	--	--	--	--	--	--	--	++
26	<i>Proteus</i> R I	++	--	--	--	--	--	--	--	++
27	Strain A	++	--	--	--	++	--	--	--	++
28	Strain B	++	--	--	--	++	--	--	--	++
29	Strain C	++	--	--	--	++	--	--	--	++
30	Strain D	++	--	--	--	++	--	--	--	++
31	Strain E	++	--	--	--	--	--	--	--	++
32	Strain F	++	--	--	--	++	--	--	--	++
33	Strain G	++	--	--	--	++	--	--	--	++
34	Strain H	++	--	--	--	++	--	--	--	++
35	Strain I	++	--	--	--	++	--	--	--	++
36	Strain J	++	--	--	--	++	--	--	--	++

A = Acid, G = Gas

PROTEOLYTIC PROPERTIES OF THE *Proteus* GROUP

As already stated Hauser subdivided the genus *Proteus* into three species—*Proteus vulgaris* and *Proteus mirabilis* which were capable of liquefying gelatine, and *Proteus zenkeri* which was not. It has also been mentioned that later he modified his statement and regarded all these as variations of the same species, namely *Proteus vulgaris*, because *Proteus zenkeri* may develop the power of liquefying gelatine, whilst the other two may lose it.

Since Hauser's time the power of liquefying gelatine has been regarded as an important property of *Proteus*, and it has been recorded by various workers on the subject—Krogus, Schnitzler, Wesenberg, Silberschmidt, Cantu, Pergola, Ford, Weil and Felix, Wenner and Rettger, Bengtson, Moltke, Taylor, and others.

Theobald Smith (1894) was able to transform a gelatine liquefying strain of *Proteus vulgaris* into a non-liquefying strain. Herter and Ten-Broeck (1911) showed that a liquefying strain of *Proteus vulgaris* which had lost its liquefying

power but remained typical in other respects could have the lost function restored by passage through a mouse Bengtson (1919) found that one of her old laboratory strains liquefied gelatine very slowly Wenner and Rettger (1919) mentioned 3 strains which according to them had lost the property of liquefying gelatine They were unable to restore the lost function by a single passage of one of these strains through a white rat Moltke (1927) examined a large number of strains and concluded that all the newly isolated strains liquefied gelatine in 1 to 4 days

In this investigation the proteolytic properties of *Proteus* were studied on gelatine and inspissated blood serum At first the usual laboratory 18 per cent gelatine was tried, but was found to give uncertain results This was then substituted with 12 per cent gelatine

Stab cultures were prepared on gelatine from a 24-hour broth culture of each strain and then kept at room temperature (16°C to 18°C) Streak cultures were made on slopes of blood serum, and incubated at 37°C Both the gelatine and serum cultures were kept under observation for 2 weeks or for shorter periods in case liquefaction took place earlier Liquefaction was noticed to start at the surface, become crateriform, and then spread down until the whole tube was involved The results have been shown in Table II

No differences were observed so far as the liquefaction of gelatine was concerned between maltose positive and maltose negative strains All the strains liquefied gelatine with the exception of Kingsbury O, Kingsbury H and Strain D, which failed to attack it Wenner and Rettger (1919) also observed loss of ability to liquefy gelatine in certain of their strains, so that the absence of this property in the presence of other characters does not necessarily exclude an organism from the *Proteus* group The Kingsbury organism is an X19 strain, which is stated by Fletcher and Lessler (1925) to have been obtained by them from Dr Neave Kingsbury It is the same strain which was supplied to the Bland-Sutton Institute of Pathology from the British National Collection of Type Cultures in 1921 As all X19 strains liquefy gelatine, evidently, therefore, this strain must have lost this faculty during its subcultural existence The same seems to be the case with Strain D which otherwise behaves, both culturally and serologically, as a member of the X19 group Kendall, Cheetham and Hamilton (1922) maintain that the ability of strains of *Bacillus proteus* (*Proteus vulgaris*) to induce liquefaction in gelatine is frequently decreased or lost through prolonged cultivation in artificial culture media, and that the loss of such power is rather a common modification of cultural and chemical properties of the typical organism The association of *Proteus vulgaris* with human lesions is, in the opinion of these authors, accompanied frequently by such a modification

The faculty of liquefying and digesting inspissated blood serum was not so constant Only 5 strains were found to possess this power, namely, 526, 6A, 752A, 78071A and 124S C The X19 strains amongst others lacked it completely On

the whole, there would seem to be an inverse relationship between maltose fermentation and serum liquefaction. All the maltose positive strains with the exception of 6A failed to liquefy blood serum. The relation between maltose negative strains and serum liquefaction was not so constant.

UREA DECOMPOSITION BY THE *Proteus* GROUP

The rôle of the *Proteus* in causing infections of the urinary tract has already been referred to, most authors have laid stress on its power of decomposing urea with the formation of ammonia. Krieger (1890), Schmitzler (1890), Hofmeister (1892), and Rovsing (1897) have referred to it. Brodmeier (1895) apparently first studied the question experimentally. He grew a *Proteus* strain in urine sterilized by heating to 100°C for 30 minutes. The amount of urea decomposed was determined by titration both before and after the cultivation of the organism. Wolf (1918) studied the urea decomposition properties of 3 *Proteus* strains in sterile urine, and found that 45 per cent of the total nitrogen of the urine was liberated. Douglas Fleming and Colebrook (1920) also emphasized the urea decomposing faculty of the *Proteus* as a diagnostic criterion. More recently Moltke (1927) studied 191 strains for urea decomposing power and found that all of them possessed this characteristic.

In this investigation owing to the difficulty of obtaining absolutely sterile samples of urine it was considered more convenient to prepare an artificial solution of urea as suggested by Moltke (1927). A 2 per cent solution of urea (Urea puriss, Merck) was prepared in 0.86 per cent salt solution, phenol red was added in the proportion of 1 in 50,000, and the pH was adjusted to 6.0. The liquid was then filtered through a Berkefeld filter to ensure sterility. A loopful from a 24-hour old agar culture was used for inoculating each strain into urea. The inoculated tubes were incubated at 37°C for 24 hours before taking the reading. All the strains included in this study with the exception of U2 gave a positive result, which was indicated by a change in coloration of the urea solution from a light yellow to a beautiful deep crimson. The X group of strains together with the Kingsbury strains were found to be the most active in this respect, the decomposition being usually apparent in their case within 4 to 6 hours.

HYDROGEN SULPHIDE PRODUCTION BY THE *Proteus* GROUP

Horowitz (1916) stated that all of her 24 strains produced hydrogen sulphide. Tanner (1917) observed similar results. Wenner and Rettger (1919) found that all of their 73 strains formed hydrogen sulphide. Likewise Bach (1921) reported that all of his 23 strains formed hydrogen sulphide. Tilley (1923) carried out a number of tests with his *Proteus* strains and found that all gave positive results. Moltke (1927) examined 194 strains and observed that all produced hydrogen sulphide.

In this investigation each strain was inoculated on to a slant of lead acetate agar and incubated at 37°C for 10 days. The formation of hydrogen sulphide was indicated by blackening of the medium. All the strains gave uniformly positive results which are shown in Table II.

INDOL PRODUCTION BY THE *Proteus* GROUP

The faculty of indol production by this genus has been pointed out by many authors, although considerable variations have been noticed in this respect.

Klhenberger (1908) found 18 strains capable of indol production out of 24. Glenn (1911) examined 7 *Proteus* strains, all of which formed indol. Herter and Ten-Broeck (1911) studied two strains which proved to be indol forming. Cantu (1911) obtained the same results on investigating 180 strains. Van Loghem and Van Loghem-Pouw (1912) in a study of 31 strains found 27 indol forming and 4 non-indol forming strains. On the basis of this study they divided the strains into two classes, namely, *Bacillus proteus indologenes* and *Bacillus proteus anindologenes*.

Berthelot (1914) found that 24 out of a total of 61 strains were indol forming. Kligler (1914) tested 5 strains and found 3 indol positive. Horowitz (1916) observed that 7 out of 24 strains examined produced indol. Stewart (1917) found 1 out of his 29 strains to be indol forming. Bengtson (1919) showed that indol production in the *Proteus* group coincided with the fermentation of maltose and saccharose. Wenner and Rettger (1919) tested 73 strains. Of these they state that 33 gave a strongly positive reaction, 36 a slightly positive and 4 a negative reaction. Moltke (1927) examined a large number of strains and found that all non-maltose fermenting strains formed no indol, and that 36 out of the 37 maltose fermenting strains formed indol.

In this study each strain was tested with Bohme's reagent. For the test each strain was grown in 1 per cent peptone water for 5 days and then 1 c.c. of the reagent was allowed to run down the side of the tube. A positive reaction was characterized by the development of a colour varying from faint pink to deep magenta. Treated in this way all *Proteus* strains were found to produce indol with the exception of the following —

Kingsbury O

Kingsbury H

526

Proteus mirabilis

Proteus 752A

Proteus 78071A

Proteus 754A

Proteus 124S C

Proteus 115A

Proteus 124S W

The relationship between maltose fermentation and indol production has been shown in Table II. It will be observed that all maltose-positive *Proteus* strains including the X strains produce indol. All the maltose negative strains fail to produce indol, the only exception being B7A2 which although maltose negative forms indol. On the whole, therefore, there would seem to be a close association between the maltose fermenting power of a *Proteus* organism and its indol forming faculty.

AGGLUTINATION REACTIONS

Many attempts have been made in the past by various workers to employ agglutination reactions as a basis for subdividing the *Proteus* group.

Wolf (1899) studied the agglutination properties of 2 *Proteus* strains with immune sera produced from these strains. The strains were agglutinable with their homologous sera, but were not mutually related.

Kheneberger (1908) studied the serological properties of a number of strains and found that some of his strains were agglutinated by the same serum but others remained unaffected.

Cantu (1911) examined 9 strains in a cross agglutination experiment and found that only two of these were homologous. He, therefore, concluded that this method could not be used for subdividing the *Proteus* group.

Van Loghem and Van Loghem-Pouw (1912) examined 31 strains and claimed that indologenic strains could be distinguished from non-indologenic strains by their agglutinative properties.

Horowitz (1916) by cross agglutination experiments saw the possibility of a serological type division of *Proteus bacilli*.

Weil and Felix (1917) investigated the relationship between the X group of strains associated with typhus fever and the *Proteus bacilli* derived from other sources. They found that none of the latter were agglutinated by typhus fever sera.

Bengtson (1919) studied a number of strains with 5 immune sera and considered that they could be subdivided into two or more groups. She found no relationship between fermentation reactions and the agglutinative properties of these strains.

Wenner and Rettger (1919) examined 73 strains by direct agglutination with 7 immune sera. With one exception all the sera agglutinated other strains besides those employed in their preparation. Some were agglutinated with more than one serum. Nineteen strains failed to be agglutinated by any of the sera. They, therefore, regard the *Proteus* group, like the streptococcus and the *Bacillus*

PLATE XLV



Fig 3 — 'O' and 'H' agglutination
The first tube shows the 'O' and the second the 'H' agglutination
The third is the control

dysenteriae group, as more or less heterogeneous, and consider that while the agglutination method may be of some value in identifying members of the *Proteus* group, negative results do not necessarily exclude an organism from the group

Moltke (1927) studied 194 strains by means of 27 immune sera produced with some of these strains. He found by agglutination experiments that swarming forms could be divided into three main groups together with several smaller groups. The sera produced with non-swarming strains did not present the same consistency of action.

In this investigation all the strains were studied by means of 4 immune sera prepared by immunizing rabbits.

These sera were prepared with the following strains —

X2' O

X19 O

X19 H

Kingsbury H

The bacillary suspensions for the agglutination tests were prepared fresh on each occasion by washing off two agar slants of a 24-hour growth of the organism concerned in 70 c.c. of a 0.86 per cent salt solution, and adjusting the density to McFarland's nephelometer tube No. 5 which is equivalent to 1,800 millions organisms per c.c. (Kolmer, 1925). Living suspensions were employed throughout these tests.

When the test was put up the tubes were well shaken and then incubated at 37°C for 2 hours. At the expiration of that period they were removed to the ice-chest and the results recorded after 24 hours. Readings were made as follows —

4+ = Organisms all precipitated, supernatant fluid perfectly clear

3+ = Organisms nearly all precipitated, supernatant fluid slightly cloudy

2+ = Organisms partially precipitated, supernatant fluid more cloudy than in 3+

+ = A perceptible precipitate of organisms, supernatant fluid cloudy

— = No precipitate or clumping. Like control without serum

The differences between 'O' and 'H' agglutination as described by Weil and Felix (1917) were well brought out during the course of the experiments. It was observed that an 'O' serum—the serum produced by injecting rabbits with an 'O' organism—agglutinated its homologous strain in fine granules, which settled down into the tube as a more or less compact sediment. On the other hand the reaction between an 'H' serum and an 'H' strain was of an altogether different order. This was shown by the development of comparatively large cotton-like coarse floccules which settled down as a large loose sediment. Plate XLV, fig. 3 serves to illustrate these differences.

The results of the agglutination tests are shown in Table IV in which the highest dilution of the sera giving a 4+ reaction with the various organisms are recorded

TABLE IV

Agglutination tests of all strains with Proteus immune sera

Serum number	Designation	Serum X2 O (Titre 5,120)	Serum X19 O (Titre 10,240)	Serum X19 H (Titre 5,120)	Serum Kingsbury H (Titre 10,240)
1	X2 O	5,120	80	0	0
2	X19 O	0	10 240	5,120	640
3	X19 H	0	5 120	5 120	320
4	X19 (N Y)	0	10 240	5 120	1,280
5	X19 (Feirer)	0	5 120	5,120	1,280
6	U2	0	0	0	0
7	Kingsbury O	0	1 280	10	5,120
8	Kingsbury H	0	1,280	1,280	10,240
9	526	0	40	640	640
10	<i>Proteus</i> I	20	1,280	2 560	2,560
11	<i>Proteus</i> II	0	160	0	40
12	<i>Proteus</i> III	0	160	0	40
13	<i>Proteus</i> V	0	0	0	0
14	<i>Proteus mirabilis</i>	20	160	2,560	640
15	<i>Proteus</i> 6A	20	640	640	1,280

Note — 'Titre' = the highest dilution giving a 4+ agglutination
 'Titre 0' = less than 4+ agglutination in a dilution of 1 20

TABLE IV—*concl'd*

Serum number	Designation	Serum $\lambda 2$ O (Titre 5,120)	Serum $\lambda 19$ O (Titre 10,210)	Serum $\lambda 19$ II (Titre 5,120)	Serum Kingsbury II (Titre 10,210)
16	<i>Proteus</i> 752A	0	40	160	80
17	<i>Proteus</i> 78071A	0	40	160	640
18	<i>Proteus</i> 754A	0	0	0	0
19	<i>Proteus</i> 91466A	0	40	320	610
20	<i>Proteus</i> 692A	0	610	610	2 560
21	<i>Proteus</i> B7A2	0	0	0	0
22	<i>Proteus</i> 124S C	0	80	80	0
23	<i>Proteus</i> 115	0	160	640	640
24	<i>Proteus</i> 115A	0	0	40	0
25	<i>Proteus</i> 124S W	0	80	80	0
26	<i>Proteus</i> R I	0	0	0	0
27	Strain A	0	5,120	5,120	0
28	Strain B	0	5,120	2,560	0
29	Strain C	0	2,560	2,560	640
30	Strain D	0	10,240	5,120	640
31	Strain E	0	80	640	20
32	Strain F	320	160	640	0
33	Strain G	0	10,240	2,560	320
34	Strain H	0	2,560	2,560	320
35	Strain I	0	2,560	2,560	160
36	Strain J	0	2,560	5,120	640

Note — 'Titre' = the highest dilution giving a 4+ agglutination
 'Titre O' = less than 4+ agglutination in a dilution of 1 20

DISCUSSION OF RESULTS

It will be seen that each immune serum agglutinates not only its homologous strain but a number of other strains as well. For example, the X19 O serum agglutinates X19 O, X19 (N Y), Strain D and Strain G to full titre. The other X19 strains, namely, X19 H, X19 (Feirer), Strains A, B, C, H, I and J give a 4+ reaction either at half titre or a quarter titre. The next group consists of X2 O, Kingsbury O, Kingsbury H, 526, *Proteus* I, *Proteus* II, *Proteus* III, *Proteus mirabilis*, 6A, 752A, 78071A, 91466A, 692A, 124S C, 115 medium active, 124S W, Strain E and Strain F. These show fairly well-marked agglutination although less than one-fourth the titre. And lastly, we have *Proteus* V, 754A, B7A2 and 115A and R I which show either very feeble or no agglutination response.

The agglutinations obtained with X19 H and Kingsbury H sera, although differing in detail, are on the same general lines. The serum X2 O which has a titre of 5,120 shows feeble agglutinative powers for only a few of the strains. It, however, gives a 4+ reaction with Strain F in a dilution of 1 in 320. The Strain F is X2 (F C) of the Pasteur Institute of Paris. It is, however, an 'H' strain, being actively motile and a rapid swimmer, whereas the immune serum has been prepared with X2 O, a non-motile and non-swarming strain.

PATHOGENICITY

The pathogenic action of the *Proteus* group in human beings has already been referred to in the preceding pages. Its pathogenicity for experimental animals has been discussed by a number of workers. Larson and Bell (1915) carried out experiments on the pathogenicity of *Proteus bacilli* and found that freshly isolated strains from human lesions were pathogenic for rabbits, rats and guinea-pigs producing abscesses or granulomatous lesions, but after cultivation under laboratory conditions such cultures produced no lesions.

Tsiklinsky (1917) states that mice, rats, rabbits and guinea-pigs are killed by subcutaneous or intra-peritoneal injections of $\frac{1}{4}$ - $\frac{1}{2}$ c c of a broth culture, especially of strains isolated from cases of infantile diarrhoea. Death usually results within 24 to 36 hours. If the animals survive abscesses result and wasting is observed.

Bengston (1919) carried out virulence tests by injecting mice with 24-hour broth cultures in 1 c c and 0.1 c c amounts. In general she found that 1 c c of broth cultures injected subcutaneously caused the death of mice within 24 hours, and 0.1 c c produced no ill effects regardless of source. Glucksmann (1899) who caused death of mice in 18 hours to 3 days by the injections of 0.1 to 0.5 c c of broth cultures of an organism isolated in a food poisoning epidemic considers the organism to have been very pathogenic for these animals. Levy (1894), Silberschmidt (1899) and Schumburg (1902) found that 0.1 to 0.5 c c of cultures were required to produce

death Wesenberg (1898) found that mice were killed with 0.2 c.c. of a 24-hour broth culture, and that less than 0.2 c.c. caused weakness and loss of appetite. Grossman (1901) isolated a *Proteus* organism from a case of peritonitis which killed mice in amounts of 0.005 c.c.

Cohn (1924) carried out a number of experiments to test the pathogenic power of these microbes. Mice were fed on broth cultures without effect, but feeding on meat soaked in broth cultures of *Proteus* for 24 hours invariably produced fatal results. Intraperitoneal injections of 2 c.c. of the broth cultures produced no ill effects.

Wenner and Rettger (1919) tested the pathogenicity of several strains by injecting 2 c.c. of saline suspensions of 24-hour agar cultures. Subcutaneous injections in rabbits produced abscesses and inflammatory conditions which lasted several months. In white rats the results varied with the strains, some causing symptoms of toxæmia and killing the animals in 18 to 24 hours, when injected by the subcutaneous route, others caused no apparent illness even when the injections were intraperitoneal.

In this investigation three strains—X19 O, X2 O and *Proteus* I—were selected for the study of their pathogenic action on white mice and rabbits. Mice were injected intraperitoneally with doses of 0.5 c.c. and 1.0 c.c. of a 48-hour broth culture of each strain. The results are shown in Table V.—

TABLE V
Results of pathogenicity experiments on mice

	Organism injected	Dose of culture	Results
Mouse No. 1	X19 O	0.5 c.c.	Died in 48 hours
Mouse No. 2	Do	1.0 c.c.	Died in 18 hours
Mouse No. 3	X2 O	0.5 c.c.	Sick for 4 to 5 days Recovered
Mouse No. 4	Do	1.0 c.c.	Do
Mouse No. 5	<i>Proteus</i> I	0.5 c.c.	Died in 24 hours
Mouse No. 6	Do	1.0 c.c.	Do

It will be observed that 0.5 c.c. of the culture was sufficient to kill the mice in 48 hours in the case of X19 O and in 24 hours in the case of *Proteus* I. One-half

and 10 c c quantities of the X2 O culture failed to destroy the animals, although it made them seriously ill

In rabbits intraperitoneal injections of 1 c c of a 48-hour broth culture of *Proteus* I and 2 c c of X19 O and X2 O were sufficient to cause the death of the animals within 4 to 5 days. Subcutaneous injections of sub-lethal doses produced localized abscesses which persisted for many months.

SUMMARY AND CONCLUSIONS

1 A review of the literature was made, discussing the distribution of organisms of the *Proteus* group and their rôle in human pathology, especially with reference to infections of the urinary tract, acute gastro-enteritis of children, meat poisoning and Weil's disease or infectious jaundice.

2 Thirty-five strains of the *Proteus* group and 1 variant strain of the *Bacillus coli* group—U2—were studied. The latter was included in this investigation on account of its agglutinability with sera obtained from cases of typhus fever.

3 A study of the morphological characters of the *Proteus* group was carried out, including the growth on solid and liquid media, staining properties, microscopic appearance, and motility. The phenomenon of swarming was studied. All the *Proteus* strains were found to be swimmers with the exception of certain strains designated as 'O' strains. Although Hauser regards swarming as an essential property of the members of this group, it is considered that lack of this character does not necessarily exclude an organism from the *Proteus* group provided it conforms to the other cultural reactions of the group.

4 The fermentation reactions of all strains were studied on a number of carbohydrates. It was found that all *Proteus* strains ferment dextrose and glycerine. None ferments lactose. All ferment saccharose including the Kingsbury strains. Twenty-four strains ferment maltose. Salicin is fermented by 17 strains including all the X strains. Levulose, galactose, xylose, arabinose and trehalose are fermented by all strains. Mannite, dulcitol, sorbitol, dextrin, starch and inulin are not fermented by any. Litmus milk exhibits a series of characteristic changes and develops a highly unpleasant odour. After a transient acidity in some cases the milk is rendered alkaline, followed by reduction of the litmus and digestion of the casein. The gas formula of the *Proteus* group was determined in dextrose broth. On the average it was $H/CO_2=3.4/1$.

5 Other reactions of the group were studied. All strains gave a negative Voges-Proskauer, a positive methyl-red test, reduced methylene-blue, reduced nitrates to nitrites, produced ammonia in peptone water and gave a positive catalase test. Likewise all strains were hæmolytic and developed an unpleasant odour in media containing peptone.

6 Proteolytic properties of the group were studied on 12 per cent gelatine and inspissated blood serum. All *Proteus* strains liquefied gelatine with the exception of 3—Kingsbury O, Kingsbury II and Strain D. No differences were observed in the liquefaction of gelatine between maltose positive and maltose negative strains. Blood serum was liquefied by 5 strains only—526, *Proteus* 6A, *Proteus* 752A, *Proteus* 78071A and *Proteus* 124S C.

7 The power of decomposing urea was exhibited by all strains. Likewise the property of producing hydrogen sulphide, indicated by the darkening of lead acetate agar, was possessed by all strains.

8 The faculty of indol production was tested by means of Bohme's reagent. All *Proteus* strains formed indol with the exception of ten. The latter group included the Kingsbury strains. In general there is a close association between the maltose fermenting power of a *Proteus* organism and its indol forming faculty.

9 The agglutinative properties of the group were studied by means of 4 rabbit immune sera. As a result of this it is concluded that —

- (a) An immune serum produced with a particular strain agglutinates not only its homologous strain but a number of other strains also.
- (b) No correlation can be established between the fermentation reactions and the agglutinating properties of the strains.
- (c) On the basis of the agglutination tests the strains can be divided into two or more groups.
- (d) The X19 strains form a group by themselves, culturally as well as serologically. The X2 strains—X2 O and Strain F—although culturally similar to the X19 group are serologically distinct.

10 The pathogenic properties of some of the strains were studied on white mice and rabbits. In general it was found that a dose of 0.5 c.c. of a 48-hour broth culture when injected intraperitoneally killed white mice in 48 hours. For the rabbits a dose of 2 c.c. was required. Subcutaneous injections in rabbits of sub-lethal doses produced localized abscesses which persisted for long periods.

Part II.

THE LABORATORY DIAGNOSIS OF TYPHUS FEVER

THE WILSON-WEIL-FELIX REACTION

THE earliest observations on the subject appear to have been made by W J Wilson in Belfast (1909, 1910) He showed that in typhus fever the blood serum often agglutinates intestinal bacilli, for example, *Bacillus coli*, *Bacillus typhosus* and especially a coliform bacillus—the *Bacillus U*—isolated from certain cases of the disease In dealing with the ætiology of typhus he pointed out that the presence of the agglutinins did not necessarily imply that the bacillus in question was of ætiological significance, and he inclined to the view that such cases were instances of secondary infection and that the agglutinins were heterologous in nature

Later, Weil and Felix (1916) investigated a group of cases thought to be enteric fever but in whom the Widal reaction against *Bacillus typhosus*, *Bacillus para-typhosus A* and *Bacillus para-typhosus B* were negative One of the cases was a Roumanian doctor who gave completely negative Widal reactions to the enteric group of organisms From his urine was isolated an organism which was agglutinated by the patient's own serum in a dilution of 1 in 200 It was then observed that the sera of nine other patients agglutinated the same organism This organism—X2—was a short, delicate, Gram negative bacillus of the *Proteus* type and was actively motile On Conrad-Dugalski medium bluish colonies developed On Endo's medium the colonies were colourless after 24 hours, but later became reddish Glucose and lactose were fermented with the production of acid and gas Milk was coagulated in 40 hours Gelatine was liquefied in 48 hours The colonies on ordinary plates closely resembles those of *Proteus vulgaris* The organism was tested against agglutinating sera for *Bacillus typhosus*, *Bacillus para-typhosus A*, *Bacillus enteritidis*, *Bacillus dysenteræ* Shiga and *Bacillus dysenteræ* Flexner, but gave negative results with all of them in dilutions of 1 in 100 Next 33 cases diagnosed clinically as typhus were tested against this organism, all of them gave a positive agglutination with the organism in dilutions varying from 1 in 25 to 1 in 500 It was observed that agglutinins for this organism developed in typhus fever at an early stage of the disease, and had already reached their height when the rash appeared about the fifth day of illness The titre remained high during the period of pyrexia and fell quickly after defervescence Two months after the end of pyrexia no patient's serum gave a higher agglutination than 1 in 25

Later Weil and Felix discovered a second strain of the same bacillus and to this strain the name X19 has been applied The essential difference between X2 and X19 is that the X19 is agglutinated up to a very much higher titre by the serum of typhus cases, i.e., up to a dilution of 1 in 2,000 or even higher The reaction,

therefore, with this strain is much more marked than with the less agglutinable original strain—X2. The X19 has been found in the urine of cases by other observers also, notably by Dienes (1917). Friedberger (1917), summing up the position of the Weil-Felix reaction, states that the test is absolutely specific for typhus fever, and agglutination is not shown by any normal serum or the serum in any other febrile disease in a greater dilution than 1 in 100 with the more agglutinable strain (X19). In cases of typhus a positive reaction is obtained in 90 per cent of all cases. The agglutination occurs in two hours at 37°C and reaches 1 in 1,000 to 1 in 2,000 or even higher. Friedberger considers that the reaction is more marked and specific than the Widal reaction in typhoid fever.

Fairley (1919), working in Palestine in 1918, confirmed the value of the Weil-Felix reaction in typhus fever. Of 65 cases of definite typhus fever 63 or 94 per cent yielded positive agglutinations. Of 120 non-typhus sera no case yielded a positive agglutination in a dilution of 1 in 20. Fairley considers that the Weil-Felix reaction in typhus fever is dependent on the presence of secondary non-specific agglutinins which have the property of agglutinating this *Proteus*-like organism.

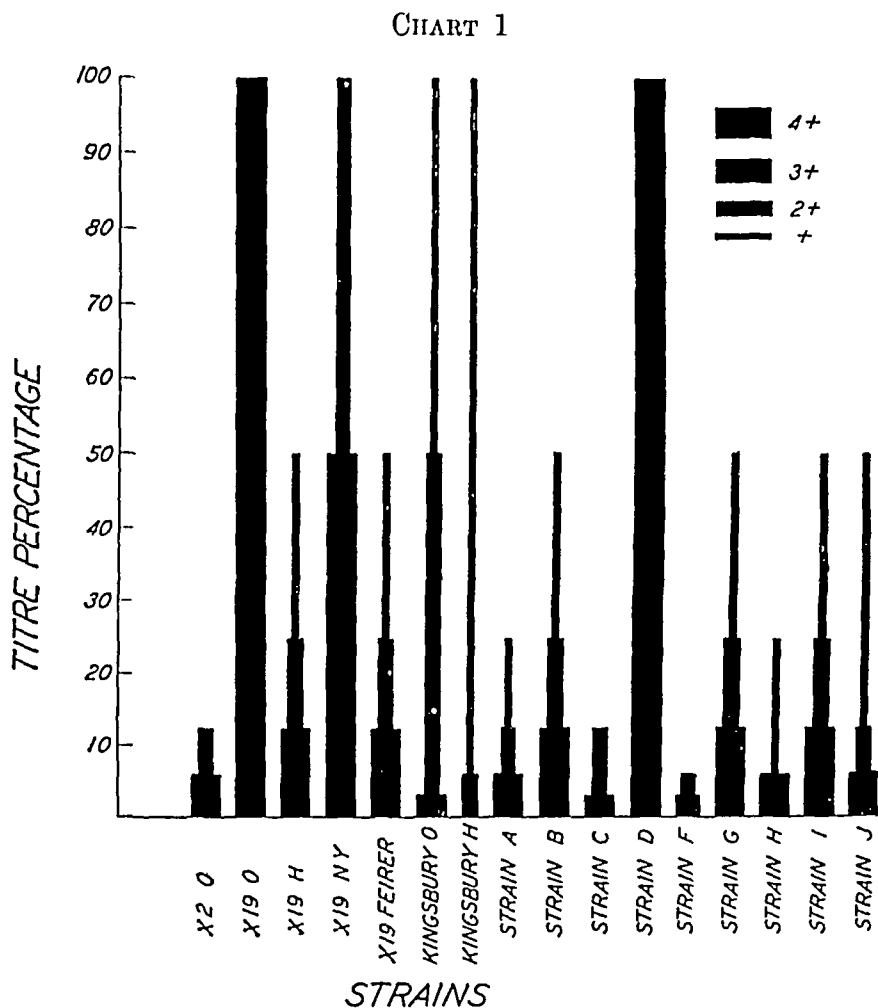
In the view of Weil and Felix the agglutination of X19 by the sera of typhus patients is essentially specific, and due to a special relation between the virus of the disease and this organism. On the other hand, Wilson, on the ground of his own experimental work with *Bacillus* U, U2, etc., considers that the X19 is only one, though the most suitable, of a number of organisms which can be used for diagnostic agglutination in typhus fever. According to the latter view, the reaction is due to the production of agglutinins in the sera of typhus patients for a variety of bacteria, and is not really specific (Wilson, 1919, 1910, 1917, 1920, 1923, 1927).

Some of the arguments brought forward against the specific action of the X strains in typhus fever are (1) the heat lability of the agglutinins found in the serum of typhus patients compared with the greater heat stability of the agglutinin in the serum of a man or animal inoculated with cultures of the X strains, (2) differences displayed with regard to the action of typhus fever agglutinins and the specific agglutinins in the serum of immunized rabbits on heated and unheated bacilli, (3) the rarity of the isolation of the X strains from the bodies of typhus fever patients, (4) the occurrence of X strains in the bodies of people who have not had typhus fever.

Weil and Felix (1917) sought to meet the objections raised as to the specificity of the X strains by showing that cultures of X19 dissociated into two varieties, one forming a raised colony without any spreading film (ohne Hauch)—‘O’ strains, and the other forming a spreading film (mit Hauch)—‘H’ strains, and by showing that the ‘O’ variety was agglutinated in the highest titre by typhus fever sera and that the agglutinins produced in the blood of rabbits inoculated with ‘O’ strains behaved like the agglutinins in typhus fever serum as to heat lability. Later (1918) they showed that the ordinary *Proteus* strains also possessed ‘O’ and ‘H’

receptors and that their 'O' receptors were in all cases distinct from those of the X strains

In this investigation an attempt was made to ascertain the relative values of the 36 strains (Table I, Part I) in the Wilson-Weil-Felix reaction for the diagnosis of typhus fever. For this purpose 4 typhus sera were obtained—3 from the National



The comparative agglutinative value of the X strains in the Wilson-Weil Felix reaction

Institute of Health (Hygienic Laboratory), Washington, D C, and one from the City General Hospital, Baltimore, Md

Living bacillary suspensions of the organisms were used in all cases. The reactions obtained with the 4 sera, although the titre varied with each, were found to be in close agreement. Table I shows the agglutination reactions with one of

the sera, and Chart 1 demonstrates the comparative agglutinative value of the X group of strains in the Wilson-Weil-Felix reaction

TABLE I
Agglutination of all strains with typhus serum

Serial number	Designation	S E R U M D I L U T I O N S						
		20	40	80	160	320	640	Control
1	X2 O	4+	4+	2+	—	—	—	—
2	X19 O	4+	4+	4+	4+	4+	4+	—
3	X19 H	4+	4+	4+	2+	+	—	—
4	X19 (N Y)	4+	4+	4+	4+	4+	2+	—
5	X19 (Feirer)	4+	4+	4+	2+	+	—	—
6	U2	4+	4+	3+	2+	+	—	—
7	Kingsbury O	4+	2+	2+	2+	2+	+	—
8	Kingsbury H	2+	2+	+	+	+	+	—
9	526	2+	2+	+	+	—	—	—
10	<i>Proteus</i> I	—	—	—	—	—	—	—
11	<i>Proteus</i> II	+	+	+	—	—	—	—
12	<i>Proteus</i> III	+	+	—	—	—	—	—
13	<i>Proteus</i> V	—	—	—	—	—	—	—
14	<i>Proteus mirabilis</i>	—	—	—	—	—	—	—
15	<i>Proteus</i> 6A	+	+	+	—	—	—	—
16	<i>Proteus</i> 752A	—	—	—	—	—	—	—
17	<i>Proteus</i> 78071A	—	—	—	—	—	—	—
18	<i>Proteus</i> 54A	2+	2+	2+	2+	2+	—	—
19	<i>Proteus</i> 91466A	—	—	—	—	—	—	—
20	<i>Proteus</i> 692A	—	—	—	—	—	—	—
21	<i>Proteus</i> B7A2	—	—	—	—	—	—	—
22	<i>Proteus</i> 124S C	—	—	—	—	—	—	—

TABLE I—*concl'd*

Serial number	Designation	SÉRUM DILUTIONS						
		20	40	80	160	320	640	Control
23	<i>Proteus</i> 115	—	—	—	—	—	—	—
24	<i>Proteus</i> 115A	—	—	—	—	—	—	—
25	<i>Proteus</i> 124S W	—	—	—	—	—	—	—
26	<i>Proteus</i> R. I	—	—	—	—	—	—	—
27	Strain A	1+	1+	2+	+	—	—	—
28	Strain B	1+	1+	4+	2+	+	—	—
29	Strain C	1+	2+	2+	—	—	—	—
30	Strain D	1+	4+	1+	1+	1+	4+	—
31	Strain E	+	—	—	—	—	—	—
32	Strain F	3+	2+	+	—	—	—	—
33	Strain G	4+	4+	4+	2+	+	—	—
34	Strain H	1+	4+	+	+	—	—	—
35	Strain I	4+	4+	4+	2+	+	—	—
36	Strain J	1+	4+	2+	+	+	—	—

As a result of the study of these reactions it is permissible to draw the following conclusions —

1 The most suitable strains for use in the Wilson-Weil-Felix reaction are the X19 O and the Strain D. I have no doubt that Strain D is also an X19 strain of the O type, since it possesses all the cultural characters of the X strains, is non-motile, lacks the swarming faculty, agglutinates up to full titre with the X19 O

immune serum, and like the X19 O is capable of removing the agglutinin from the X19 O immune serum

2 Of the other X19 strains, including the Strains A, B, C, G, H, I and J, X19 (N Y)—the strain carried by the New York State Health Department—is the next best. It shows a 4+ agglutination in a dilution of 1 in 320, and a 2+ reaction in 1 in 640 which is the titre of the serum. The other X19 strains give a final 4+ reaction in different dilutions varying from 1 in 20 to 1 in 80.

3 The X2 strains—X2 O and Strain F—are, on the whole, much less agglutinable than most of the X19 strains. X2 O gives a 4+ reaction up to 1 in 40 only.

4 Of the Kingsbury strains, which are stated by Fletcher and Lesslar (1926) to be more agglutinable than X19 in certain cases of tropical typhus, the Kingsbury O seems to show somewhat better results, giving a 4+ reaction up to 1 in 20 and a + reaction up to 1 in 640.

5 Other strains—U2, 526, *Proteus* II, *Proteus* III, *Proteus* 6A and *Proteus* 754A—also possess feebler agglutinative properties.

6 It is worthy of note that of the five known strains derived from lower animals—*Proteus* 124S C, *Proteus* 115, *Proteus* 115A, *Proteus* 124S W and *Proteus* R I—none exhibits any trace of agglutinability with typhus serum.

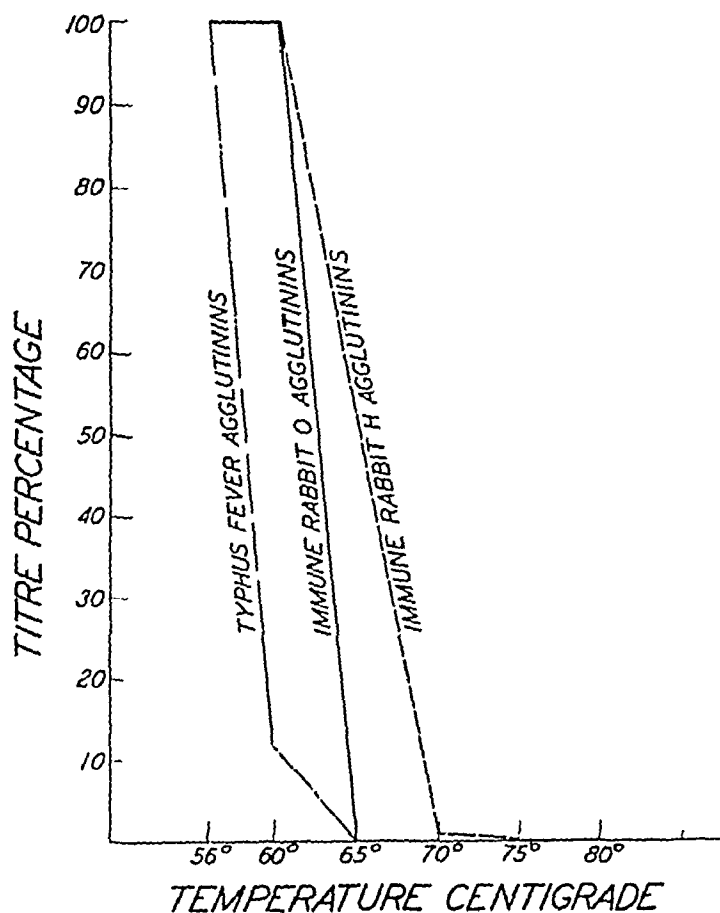
*The effect of heat on typhus fever agglutinins and the immune rabbit
'O' and 'H' agglutinins*

Weil and Felix (1917) have shown that cultures of X19 can be dissociated into two varieties, one the ordinary swarming or the 'H' form and the other the non-swarming variety or the 'O' form. They have also found that the 'O' variety is agglutinated in the highest titre by the sera of typhus fever patients, and that such sera invariably contain the 'O' agglutinins. *Proteus* X19, however, also reacts with the 'H' agglutinins and it is well known that such agglutinins are occasionally met with in the sera of individuals suffering from various non-typhus diseases like cystitis, nephritis, otitis, etc., due to an existing or a previous infection with organisms of the *Proteus* group, and it has also been shown in the preceding pages that a *Proteus* immune serum is usually capable of agglutinating not only its homologous strain but a number of other strains as well. The matter becomes, therefore, of great practical importance, and it is conceivable that such a contingency may constitute an important source of error in the interpretation of the Wilson-Weil-Felix reaction. To obviate this difficulty Weil and Felix (1917) and Felix and Olitzki (1929) made a comparative study of the typhus and immune agglutinins. They found that the agglutinins produced in the blood of rabbits inoculated with an 'O' strain of *Proteus* X19 behaved as to heat lability like the agglutinins in typhus fever serum. Heating the serum to 65°C to 70°C for 40 minutes destroyed the 'O' agglutinins, but the 'H' agglutinins although reduced

in titre were not destroyed. The question has been investigated by various other workers also, but the procedure has not yet yielded uniform results in all cases. It was, therefore, considered desirable to undertake a study of this problem during the course of this investigation.

A typhus serum obtained from a convalescent case of the disease and X19 O and X19 H immune sera prepared by injecting rabbits were diluted 1 in 20 with

CHART 2



The effect of temperature in 1 hour on typhus fever agglutinins and the immune rabbit 'O' and 'H' agglutinins

sterile 0.86 per cent salt solution and heated to 56°C, 60°C, 65°C, 70°C, and 75°C for different periods of time, varying from quarter of an hour to 3 hours, when the agglutination tests were set up with the living homologous strains in the case of the immune sera and with a living X19 O culture in the case of typhus serum.

The differences are clearly shown in Table II which gives the results after heating for 1 hour. The same is illustrated graphically in Chart 2.

TABLE II

Comparison of 'O' and 'H' agglutinins in immune rabbit sera and 'O' agglutinins in the serum of a typhus fever patient

Serum	Agglutination with strain	Type of agglutination	Titre of agglutination and the percentage that remains after heating the sera in a dilution of 1 in 20 for 60 minutes at				
			56°	60°	65°	70°	75°
Rabbit serum X19 O	X19 O	'O'	$\frac{1}{100} \frac{10,240}{100}$	$\frac{1}{100} \frac{10,240}{100}$	$< \frac{1}{0} \frac{20}{0}$	$< \frac{1}{0} \frac{20}{0}$	$< \frac{1}{0} \frac{20}{0}$
Rabbit serum X19 H	X19 H	'H'	$\frac{1}{100} \frac{5,120}{100}$	$\frac{1}{100} \frac{5,120}{100}$	$\frac{1}{50} \frac{2,560}{50}$	$\frac{1}{0.4} \frac{20}{0.4}$	$< \frac{1}{0} \frac{20}{0}$
Typhus fever patient's serum	X19 O	'O'	$\frac{1}{100} \frac{640}{100}$	$\frac{1}{12.5} \frac{80}{12.5}$	$< \frac{1}{0} \frac{20}{0}$	$< \frac{1}{0} \frac{20}{0}$	$< \frac{1}{0} \frac{20}{0}$

It is thus apparent that the 'O' agglutinins in typhus fever serum are more thermolabile than the 'H' agglutinins in the rabbit immune serum, having been greatly reduced in titre at 60°C and completely destroyed at 65°C in one hour. Of the immune agglutinins—produced in the blood of rabbits by inoculation with cultures of *Proteus* X19—the 'O' agglutinins unlike the 'O' agglutinins in typhus fever serum show no reduction in titre at 60°C, although like the latter they, too, are destroyed at 65°C in one hour. The 'O' agglutinins in typhus fever serum are thus considerably less heat-resistant than the 'O' agglutinins in the rabbit immune serum. The 'H' agglutinins in the immune rabbit serum, on the other hand, are much more thermostabile, a temperature of 75°C for one hour being necessary for their inactivation.

The differences in the heat resistance of the 'O' and 'H' agglutinins may be made use of in routine laboratory practice for distinguishing between typhus fever and infections caused by organisms of the *Proteus* group. Wadsworth (1927) states that sera from typhus cases when heated to from 56°C to 58°C generally lose their ability to agglutinate cultures of *Proteus* X19 and may be thus differentiated from sera from cases of *Proteus* infection or those from animals inoculated with cultures of *Bacillus proteus*. In view of the results obtained in this study it would

be necessary to carry out the Wilson-Weil-Felix reaction with the serum heated to 65°C for one hour, in addition to the usual test with the unheated serum. An evidence of thermolability would be regarded in favour of a diagnosis of typhus fever.

The comparative value of different kinds of bacillary suspensions of Proteus X19 O in the Wilson-Weil-Felix reaction

Some workers have recommended suspensions of X19, killed and preserved with phenol or formaline, for use in the Wilson-Weil-Felix reaction, others have noted the partial inhibition of the reactions in the presence of the antiseptic and recommended the use of living cultures as being more sensitive. To avoid the inconvenience associated with the use of living cultures Bien (1924) prepared an alcoholic suspension. This has been reported as satisfactory by Felix (1930) who suggests its use either as a control with the living culture or as the only reagent when the latter is not available. Gardner (1929) has used the alcoholic method for the preparation of 'O' suspensions of *Bacillus typhosus* for the Widal test and reports satisfactory results.

In this investigation the behaviour of 5 types of bacillary suspensions—the living, the heat killed, the formalized, the carbolized and the alcoholic—was studied both with respect to typhus serum and the immune rabbit X19 O serum. The heat killed suspension was prepared by heating a saline suspension of a 24-hour agar culture of X19 O to 60°C for 1 hour. The formalized was prepared by the addition of 0.3 per cent of formaldehyde to a saline suspension of the organism, and keeping it in the ice-chest for three days. In the carbolized suspension the organisms were suspended in normal salt solution containing 1 per cent phenol and stored in the ice-chest for three days. The alcoholic suspension was prepared by inoculating a Blake bottle of agar with 1 c.c. of a 24-hour broth culture of the organism—X19 O—and incubating for 24 to 48 hours at 37°C. The growth was washed off with 40 c.c. of normal salt solution containing 0.5 per cent phenol, and 20 c.c. of absolute alcohol were gradually added, while the mixture was stirred with a glass rod. The suspension was allowed to stand for 24 hours at 37°C for the deposition of solids. At the end of that time the supernatant fluid was poured off into a sterile bottle. At the time of use the density of the different suspensions was adjusted to McFarland's nephelometer tube No. 5.

The results obtained by using the different kinds of bacillary suspensions with typhus serum and the rabbit immune X19 O serum are shown in Table III.

These results indicate that a living saline suspension of *Proteus* X19 O is the most sensitive for the Wilson-Weil-Felix reaction. Next in order of sensitivity are the alcoholic, the carbolized, the heat killed and, lastly, the formalized which is the least sensitive of all.

For use with immune sera the living, the alcoholic, and the heat killed suspensions are about equally satisfactory. Next comes the formalized and last of all the carbolized which is comparatively much less sensitive.

It may be mentioned here that the results of agglutinations with the alcoholic suspension are a little difficult to read, the end points of the reactions being not so well defined and clear cut as with the living suspension.

It is, therefore, obvious that for routine laboratory use in the diagnosis of typhus fever a living saline suspension of a 24-hour agar culture of *Proteus* X19 O is the most suitable one to employ. Of the preserved suspensions reliable results can only be obtained with the alcoholic suspension.

SUMMARY AND CONCLUSIONS

1 The history of the Wilson-Weil-Felix reaction was discussed and the reaction was carried out with the sera of 4 typhus patients on all the strains. It was found that X19 O and Strain D were the most suitable for this purpose.

2 The effect of heat on the 'O' agglutinins in typhus fever serum and the 'O' and 'H' agglutinins in immune rabbit sera was studied. It was found that the 'O' agglutinins in typhus fever serum unlike the 'O' agglutinins in immune rabbit serum are greatly reduced in titre at 60°C in 1 hour. Both are, however, destroyed at 65°C. The 'H' agglutinins in the immune rabbit serum, on the other hand, are much more heat resistant, a temperature of 75°C being necessary for their complete inactivation. To distinguish between typhus fever agglutinins and agglutinins due to infections with organisms of the *Proteus* group it is suggested that the Wilson-Weil-Felix reaction be carried out with the unheated serum and the serum heated to 65°C for one hour. An evidence of thermolability to be considered in favour of a diagnosis of typhus fever.

3 A comparative study of the value of different kinds of bacillary suspensions of *Proteus* X19 O in the Wilson-Weil-Felix reaction and for purposes of agglutination with rabbit immune sera was made. A living saline suspension of a 24-hour agar culture of the organism is considered the most suitable for routine laboratory use in the diagnosis of typhus fever. Of the preserved suspensions the alcoholic is the only one which gives reliable results.

ACKNOWLEDGMENTS

I owe a debt of gratitude to Dr William W Ford, Professor of Bacteriology, and Dr Samuel R Damon, Associate Professor of Bacteriology, School of Hygiene and Public Health, Johns Hopkins University, for advice during the course of this investigation. I have also to express my indebtedness to Dr Kenneth F Maxey, Professor of Hygiene and Public Health, University of Virginia, Dr C L Williams, Surgeon, United States Public Health Service, Dr Linda B Lange, Associate Professor of Bacteriology, Dr Cahista P Elhot, Associate in Bacteriology,

Dr William A. Fencl, Associate in Bacteriology, School of Hygiene and Public Health, Johns Hopkins University, and Dr Cornelius A. Periy, Director, Bacteriological Laboratories, State Department of Health, Maryland, for the supply of various strains of *Proteus vulgaris*.

Lastly, my thanks are due to Dr R. R. Spencer, Surgeon, United States Public Health Service, the National Institute of Health, Washington, D. C., and to Dr W. A. Buice, Assistant Professor of Bacteriology, University of Oklahoma, for the supply of sera from patients of typhus fever.

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BASAL METABOLISM OF YOUNG COLLEGE STUDENTS, MEN AND WOMEN, IN MADRAS

BY

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ASSISTED BY

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[Received for publication July 30, 1931]

It has been the practice in India, to use, in the determination of basal metabolic rates, one or the other of the normal standards of Aub and Du Bois, Harris-Benedict and Dreyer, for comparison, and pronounce the rates to be above or below normal. These standards were based entirely on the determinations made in America, and E F Du Bois in his 'Basal Metabolism in Health and Disease' observes 'we know rather little about the differences in the metabolism of different races and, as a matter of fact, almost all the determinations that we have used have been made in America or Germany.'

Having regard to the climate of India, and to the well recognized differences in race, general physique and diet of the people, it is of great importance that basal metabolic determinations should be made exclusively in this country, in different areas on a number of normal men and women of different ages, and that standards applicable to India be laid down, so that real variations in disease and other abnormal conditions may be known. An individual in this country may show a basal metabolic rate which varies plus or minus 10 per cent from the predicted normal according to the western standard, and yet be normal.

There seems to be no unanimity in the adoption of any particular standard even in the West. In America Aub-Du Bois' standard is favoured. In Germany and other parts of the continent Harris-Benedict's prediction tables are preferred, and in England Dreyer's figures are employed as a rule. August Krogh observed in 1923 'the Harris-Benedict prediction tables for basal metabolism are, owing to their statistical nature, less reliable for persons of exceptional build or higher age. The Du Bois' method gives results which on an average are too high (4 per cent or

more), but less likely to fail on exceptional subjects'. According to E F Du Bois, the Dreyer's method possesses no material advantage over the Harris-Benedict's, and reports on adults are better made according to both the Sage and Harris-Benedict standards. Grafe, one of the most experienced investigators, averaged the two. August Krogh (1925) modified Aub-Du Bois figures by making a uniform reduction of 6 per cent. J T King, in his monograph on basal metabolism, observes that workers in metabolism would do better to select normal standards for their use, from observations made under conditions as nearly as possible similar to those under which they plan to work.

So far as I could gather from the literature available, very little work has been done in India to indicate the probable standards of basal metabolism for Indians. Mukherjee in 1926, in a brief note, reported in the *Journal of American Medical Association* that the basal metabolism of fifteen Bengali medical students averaged 9 per cent lower than the generally accepted standards, and again he and Gupta report in the *Indian Journal of Medical Research* (January, 1931) that the basal metabolism of eighteen Bengali men from 20 to 29 years of age averaged 13.3 per cent lower than the Du Bois standards. Sokhey in 1927 reported at the Seventh Congress, Far Eastern Association of Tropical Medicine, that fifteen of his subjects at Bombay showed a basal metabolic rate 10 to 23 per cent lower than the Du Bois standards.

So, with a view to make a beginning in the finding of normal standards for South India, data were collected, on 76 men and women of ages ranging from 18 to 25, coming from different districts. The subjects were all medical students who were having or had had a course of lectures on the subject of metabolism, and so were acquainted with the apparatus and the conditions necessary for an accurate determination. It is said that the science of metabolism has been founded on the very bodies of medical students and physiologists and, as a matter of fact, they are the persons who are readily available for such investigations and serve as normal controls.

The basal metabolism determinations were made in the mornings between 8 and 10 A M, 14 to 18 hours or even 20 hours in a few cases, after the last meal, the subjects having had according to instructions an evening meal at 6 P M or earlier and no breakfast next morning till the basal metabolism determinations were made. All the subjects were either carried by car from their residences to the Laboratory in the morning or were asked to spend the night at a convenient place where the determinations could be done in the morning. Before each determination, complete muscular relaxation by lying down on improvised cots for 30 to 45 minutes was insisted upon. It was observed that there was no appreciable difference in the results whether the method adopted, to secure the subjects for the determinations was ambulatory or retention overnight at the place where the tests were made, so long as 30 to 45 minutes' rest was given before the determination.

Three determinations were made in each case on two different days, the second and the third having been made on the same day, as there was difficulty in securing the subjects under basal conditions on more than two different days

Apparatus used was the latest model of Benedict-Roth's with rubber flutter valves and with the Collin's Kymograph attachment. In this apparatus, the bell has a standard capacity of 20.73 c.c. per mm. of height. This amount of oxygen (at the average calorific value of 4.825 Cal. per litre with R. Q. 0.82) equals 0.1 Cal. Therefore 1 mm. fall in 6 minutes represents 1 Cal. per hour, subject to the normal standard corrections for temperature, pressure and moisture. Tests for leaks and unabsorbed carbon dioxide in the bell were often made.

The body surface in all cases was computed from the charts devised by Aub-Du Bois based on height-weight formula

$$A = W^{0.425} \times H^{0.725} \times 71.84$$

In a few cases (Table X), the old Meeh's formula was used just for comparison with the results obtained by the more accurate Du Bois' formula. It is found that the Meeh's formula [$A = 0.123 X^3$ (body-weight in kg.)²] gives more constant results, though a larger body surface and a lower basal metabolic rate per sq. m. per hour.

About 230 basal metabolism determinations were made on 76 men and women (three tests on each subject) of 18 to 25 years of age and the results are embodied in Tables I and II. Table I represents the minimum of three observations on each subject with the deviations from the Aub and Du Bois, Harris-Benedict and Dreyer's standards. Table II represents the average of the three observations on each subject with the deviations from the standards.

TABLE I

Basal metabolic rate of normal young men and women

(Minimum of three readings as a rule)

Number of subject	Age Years	Sex	HEIGHT IN CM		Weight in kilo	Body surface sq. m	Basal metabolic rate per sq. m. per hour Cal.	DEVIATIONS OF ACTUAL FROM PREDICTED PER CENT		
			Standing	Sitting				Aub-Du Bois' standard	Harris Bene- dict's standard	Dreyer's standard
5	18	M	167		52.5	1.59	38.2	- 6.9	- 3.1	- 5.5
16	18	M	166	89	57.0	1.63	39.3	- 4.2	- 0.6	- 4.0
17	18	M	152	76	36.5	1.27	42.0	2.5	6.0	- 0.2
33	18	M	172	88	54.0	1.63	39.6	- 3.3		- 0.5

J, MR

TABLE I—*contd*

Number of subject	Age Years	Sex	HEIGHT IN CM		Weight in kilo	Body surface sq m	Basal metabolic rate per sq m per hour Cal	DEVIATIONS OF ACTUAL FROM PREDICTED PER CENT		
			Standing	Sitting				Aub Du Bois' standard	Harris-Benedict's standard	Dreyer's standard
13	19	M	175	91	46.0	1.55	44.7	8.9	15.2	16.3
15	19	M	156	83	50.2	1.48	39.4	-3.9	-0.5	-6.5
22	19	M	163	85	48.0	1.49	31.4	-23.2	-20.4	-22.8
40	19	M	166	88	54.1	1.59	35.8	-12.6	-9.2	-11.5
47	19	M	178	91	45.4	1.56	35.0	-14.8	-9.9	-7.8
48	19	M	170	88	49.1	1.56	31.4	-23.0	-19.5	-20.0
1	20	M	170	89	56.2	1.64	38.0	-3.8	-3.0	-3.8
8	20	M	164	84	56.0	1.60	41.1	4.2	4.0	1.0
12	20	M	167	90	50.2	1.56	37.5	-5.5	-3.7	-5.4
26	20	M	169	86	50.8	1.58	36.6	-9.7	-7.2	-8.6
27	20	M	177	86	52.7	1.64	39.9	0.8	2.1	3.8
28	20	M	159	81	44.5	1.42	31.5	-20.2	-19.8	-22.8
29	20	M	174	92	53.2	1.56	38.3	-2.6	-5.3	-4.6
30	20	M	171	88	54.0	1.63	34.7	-12.0	-11.3	-11.6
43	20	M	171	83	60.4	1.70	36.5	-7.5	-7.6	-8.0
6	21	M	161	85	43.3	1.42	35.6	-10.8	-9.2	-12.2
11	21	M	166	87	44.3	1.46	30.8	-22.1	-20.7	-22.1
19	21	M	166	89	53.0	1.58	34.4	-13.1	-12.0	-13.7
20	21	M	167	89	54.0	1.61	35.3	-10.5	-9.0	-10.6
25	21	M	185	98	72.0	1.95	36.6	-7.5	-7.0	-3.1
34	21	M	164	83	54.0	1.58	35.6	-10.1	-9.1	-11.7
36	21	M	159	86	51.3	1.52	38.6	-2.1	-0.8	-5.1
38	21	M	164	85	48.7	1.51	38.3	-2.6	-1.5	-4.0
45	21	M	170	89	53.1	1.61	32.5	-17.6	-16.4	-16.9

TABLE I—contd

Number of subject	Age Years	Sex	HEIGHT IN CM		Weight in k.ilo	Body surface sq m	Basal metabolic rate per sq m per hour Cal	DEVIATIONS OF ACTUAL FROM PREDICTED PER CENT		
			Standing	Sitting				Aub Du Bois' standard	Harris-Benedict's standard	Dreyer's standard
50	21	M	163	84	49.6	1.52	32.8	-17.0	-17.5	-19.6
53	21	M	168	82	45.4	1.50	32.0	-18.9	-17.2	-17.9
59	21	M	171	88	69.5	1.81	38.2	-3.1	-4.2	-4.2
10	22	M	165	87	48.8	1.52	34.5	-12.1	-10.7	-12.6
14	22	M	166	88	55.7	1.62	32.9	-16.6	-15.4	-16.8
21	22	M	166	84	50.7	1.56	32.4	-19.0	-15.9	-17.3
23	22	M	170	85	54.8	1.63	38.3	-3.1	-1.2	-2.0
35	22	M	159	85	45.4	1.44	36.8	-6.8	-5.0	-8.6
41	22	M	172	84	47.3	1.54	34.2	-13.4	-10.1	-10.6
51	22	M	172	94	56.8	1.66	32.3	-18.1	-17.2	-17.3
52	22	M	161	82	50.0	1.50	33.2	-16.0	-15.0	-18.0
54	22	M	164	86	54.1	1.58	33.2	-16.0	-15.1	-17.2
60	22	M	180	90	57.7	1.73	29.5	-25.4	-24.0	-21.9
32	22	M	165	86	50.8	1.55	36.7	-7.0	-4.8	-7.9
2	23	M	170	85	50.7	1.58	32.6	-17.6	-15.2	-15.3
3	23	M	164	87	47.0	1.49	33.6	-14.6	-12.6	-14.4
4	23	M	160	87	62.0	1.65	40.2	1.8	1.6	-1.6
24	23	M	173	93	61.0	1.73	30.0	-24.5	-23.6	-23.0
31	23	M	163	84	50.8	1.53	35.2	-11.0	-9.1	-11.7
37	23	M	160	87	52.0	1.53	41.6	6.7	7.0	3.1
39	23	M	173	88	65.0	1.78	32.8	-17.0	-16.2	-16.4
42	23	M	161	84	46.8	1.47	35.2	-10.3	-8.2	-10.4
44	23	M	170	84	50.9	1.58	34.0	-13.4	-11.5	-11.5
46	23	M	165	81	57.2	1.62	34.4	-13.0	-12.0	-13.8

TABLE I—concl'd

Number of subject	Age Years	Sex	HEIGHT IN CM		Weight in kilo	Body surface sq m	Basal metabolic rate per sq m per hour Cal	DEVIATIONS OF ACTUAL FROM PREDICTED PER CENT		
			Standing	Sitting				Aub Du Bois' standard	Harris-Benedict's standard	Dreyer's standard
55	23	M	163	87	63.2	1.68	30.1	-23.6	-23.6	-25.2
56	23	M	176	85	61.4	1.75	30.0	-23.8	-22.7	-21.6
57	23	M	174	92	80.9	1.96	40.6	2.8	0.9	3.2
61	23	M	174	85	46.4	1.54	34.0	-13.9	-11.2	-9.7
7	24	M.	169	84	51.5	1.59	32.1	-19.0	-16.1	-16.3
9	24	M	161	84	52.0	1.53	29.4	-25.6	-24.1	-26.5
18	24	M	166	85	60.2	1.68	39.1	-1.0	1.0	-0.4
49	24	M	159	84	46.4	1.44	39.1	-1.0	1.0	-2.7
58	25	M	157	81	50.0	1.48	36.5	-7.7	-5.2	-9.5
65	19	F	159	79	51.8	1.51	31.6	-16.5	-15.1	-16.0
66	20	F	153	80	40.5	1.33	26.8	-27.5	-30.4	-28.4
62	21	F	154	79	46.4	1.41	28.5	-23.0	-24.8	-24.2
64	21	F	150	80	43.7	1.35	34.0	-8.5	-13.9	-12.5
67	21	F	140	71	37.3	1.20	30.5	-17.0	-24.9	-23.0
68	21	F	161	83	37.3	1.34	29.8	-19.0	-21.0	-16.1
63	22	F	150	77	53.2	1.47	29.8	-19.0	-21.2	-22.0
69	22	F	150	77	46.4	1.38	31.5	-15.0	-17.6	-17.3
76	22	F	156	81	50.9	1.49	32.6	-12.0	-12.1	-12.0
70	23	F	150	74	35.0	1.23	30.2	-20.0	-19.3	-17.3
72	23	F	150	76	49.1	1.42	35.6	-3.5	-5.8	-5.8
73	23	F	151	79	56.4	1.51	31.0	-16.0	-17.7	-19.0
74	23	F	162	83	50.0	1.52	30.0	-19.0	-17.5	-16.2
71	23	F	163	86	46.4	1.47	29.5	-20.0	-19.3	-17.3
75	24	F	150	77	38.7	1.27	34.2	-7.0	-12.1	-8.8

TABLE II

Basal metabolic rate of normal young men and women

(Average of three readings as a rule)

Number of subject	Age Years	Sex	HEIGHT IN CM		Weight in kilo	Body surface sq m	Basal metabolic rate per sq m per hour Cal	DEVIATIONS OF ACTUAL FROM PREDICTED PER CENT		
			Standing	Sitting				Aub-Du Bois standard	Harris-Benedict's standard	Droeyer's standard
5	18	M	167		52.5	1.59	40.5	-1.7	2.1	-0.3
16	18	M	166	89	57.0	1.63	41.3	0.5	4.2	0.8
17	18	M	152	76	36.5	1.27	42.6	4.1	7.6	1.4
33	18	M	172	88	54.0	1.63	40.6	-1.3	2.7	2.1
13	19	M	175	91	46.0	1.55	45.9	11.9	18.1	19.6
15	19	M	156	83	50.2	1.48	39.6	-3.3		-6.0
22	19	M	163	85	48.0	1.49	33.6	-18.0	-15.0	-17.5
40	19	M	166	88	54.1	1.59	36.8	-10.2	-6.9	-9.0
47	19	M	178	91	45.4	1.56	39.5	-3.6	2.0	4.2
48	19	M	170	88	49.1	1.56	33.5	-18.2	-14.0	-14.7
1	20	M	170	88	56.2	1.64	38.5	-2.5	-2.0	-2.5
8	20	M	164	84	56.0	1.60	42.7	8.0	7.9	4.8
12	20	M	167	90	50.2	1.56	42.0	6.0	8.0	6.1
26	20	M	169	86	50.8	1.58	36.0	-9.8	-7.3	-8.5
27	20	M	177	86	52.7	1.64	41.3	4.1	5.7	7.1
28	20	M	159	81	44.5	1.42	34.9	-11.4	-11.1	-14.4
29	20	M	174	92	53.2	1.56	38.8	-1.2	-3.8	-3.3
30	20	M	171	88	54.0	1.63	35.2	-10.7	-10.1	-10.4
43	20	M	171	83	60.0	1.70	39.0	-1.7	-1.7	-2.0

TABLE II—*contd*

Number of subject.	Age Years	Sex	HEIGHT IN CM		Weight in kilo	Body surface sq m	Basal metabolic rate per sq m per hour Cal	DEVIATIONS OF ACTUAL FROM PREDICTED PER CENT		
			Standing	Sitting				Aub-Du Bois' standard	Harris-Benedict's standard	Dreyer's standard
6	21	M	161	85	111	1.48	37.0	-6.7	-5.0	-8.1
11	21	M	166	87	44.3	1.46	34.2	-11.0	-12.5	-14.0
19	21	M	166	89	53.0	1.58	34.8	-12.0	-10.9	-12.7
20	21	M	167	89	51.0	1.61	35.9	-9.0	-7.5	-9.1
25	21	M	185	98	72.0	1.95	37.2	-5.9	-5.4	-1.4
34	21	M	164	83	51.0	1.58	37.9	-4.3	-3.3	-6.0
36	21	M	159	86	51.3	1.52	39.3	-0.6	0.4	-3.7
38	21	M	164	85	48.7	1.51	39.3	-0.6	0.7	-2.0
45	21	M	170	89	53.1	1.61	35.2	-11.0	-9.6	-10.2
50	21	M	163	84	49.6	1.52	37.1	-6.1	-4.8	-7.5
53	21	M	168	82	45.4	1.50	32.5	-17.5	-15.6	-16.5
59	21	M	171	88	69.5	1.81	40.8	3.1	2.0	2.0
10	22	M	165	87	48.9	1.52	37.4	-5.2	-3.5	-5.5
14	22	M	166	88	55.7	1.62	35.2	-11.0	-9.5	-11.3
21	22	M	166	84	50.7	1.56	34.6	-13.6	-10.2	-11.8
23	22	M	170	85	54.8	1.63	40.0	1.3	3.0	2.3
35	22	M	159	85	45.4	1.44	40.0	1.3	3.7	-0.3
41	22	M	172	84	47.3	1.54	37.5	-5.0	-2.6	-2.1
51	22	M	172	94	56.8	1.66	32.8	-16.7	-15.8	-16.0
52	22	M	161	82	50.0	1.50	34.8	-12.0	-11.0	-14.1
54	22	M	164	86	54.1	1.58	34.3	-13.0	-12.1	-14.4
60	22	M	180	90	57.7	1.73	31.1	-21.2	-19.7	-17.5
32	22	M	165	86	50.8	1.55	37.5	-5.0	-0.4	-3.6

TABLE II—*contd*

Number of subject	Age Years	Sex	HEIGHT IN CM		Weight in kilo	Body surface sq m	Basal metabolic rate per sq m per hour Cal	DEVIATIONS OF ACTUAL FROM PREDICTED PER CENT		
			Standing	Sitting				Aub Du Bois' standard	Harris-Benedict's standard	Dreyer's standard
2	23	M	170	85	50.7	1.58	33.5	-15.4	-13.0	-13.1
3	23	M	164	87	47.0	1.49	35.2	-10.7	-8.4	-10.5
4	23	M	160	87	62.0	1.65	41.9	6.1	6.0	2.8
24	23	M	173	93	61.0	1.73	35.4	-10.3	-9.1	-8.3
31	23	M	163	84	50.8	1.53	35.5	-10.1	-8.3	-10.8
37	23	M	160	87	52.0	1.53	43.3	10.8	11.1	7.2
39	23	M	173	88	65.0	1.78	36.6	-7.4	-6.3	-5.3
42	23	M	161	84	46.8	1.47	37.3	-5.8	-3.4	-6.4
44	23	M	170	84	50.9	1.58	36.6	-7.3	-5.2	-5.2
46	23	M	165	81	57.2	1.62	36.1	-8.6	-7.7	-9.6
55	23	M	163	87	63.2	1.68	33.3	-15.8	-15.8	-17.8
56	23	M	176	85	61.4	1.75	31.1	-21.2	-20.0	-18.7
57	23	M	174	92	80.9	1.96	44.1	11.6	9.6	12.0
61	23	M	174	85	46.4	1.54	34.7	-12.4	-9.7	-8.2
7	24	M	169	84	51.5	1.59	33.8	-14.7	-11.7	-12.0
9	24	M	161	84	52.0	1.53	33.5	-15.2	-12.0	-16.3
18	24	M	166	85	60.2	1.68	39.7	0.4	2.1	1.0
49	24	M	159	84	46.4	1.44	39.5		2.1	-1.7
58	25	M	157	81	50.0	1.48	37.4	-5.4	-2.8	-7.2
65	19	F	159	79	51.8	1.51	31.6	-16.5	-15.1	-16.0
66	20	F	153	80	40.5	1.33	28.6	-22.5	-25.7	-23.5
62	21	F	154	79	46.4	1.41	29.1	-21.1	-23.2	-22.6

TABLE II—concl'd

Number of subject	Age Years	Sex	HEIGHT IN CM		Weight in kilo	Body surface sq m	Basal metabolic rate per sq m per hour Cal	DEVIATIONS OF ACTUAL FROM PREDICTED PER CENT		
			Standing	Sitting				Aub-Du Bois' standard	Harris Benedict's standard	Dreyer's standard
64	21	F	150	80	43.7	1.35	34.6	-6.6	-10.5	-9.9
67	21	F	140	71	37.3	1.20	31.8	-13.8	-21.8	-19.0
68	21	F	161	83	37.3	1.34	33.7	-8.7	-10.8	-5.2
63	22	F	150	77	53.2	1.47	31.4	-19.3	-21.2	-22.2
69	22	F	150	77	46.4	1.38	33.1	-10.4	-13.6	-13.2
76	22	F	156	81	50.9	1.49	33.8	-8.9	-9.2	-9.0
70	23	F	150	74	35.0	1.23	31.5	-15.0	-19.9	-13.6
71	23	F	163	86	46.4	1.47	31.6	-14.9	-13.8	-11.7
72	23	F	150	76	49.1	1.42	35.7	-3.7	-6.3	-6.3
73	23	F	151	79	56.4	1.51	31.7	-14.2	-16.0	-17.2
74	23	F	162	83	50.0	1.52	30.0	-19.0	-17.5	-16.2
75	24	F	150	77	38.7	1.27	35.8	-3.1	-8.0	-3.6

Tables III and IV represent the summaries of the results shown in Tables I and II for men and women according to ages and, in addition, the deviation from Krogh's modification of Du Bois' standard. The results of numbers 8, 13 and 37 are not included in the summaries owing to the wide deviations from the calculated average, probably caused by some unsuspected illness or a high protein diet.

It would be seen that the figures in Table III, based on the minimum of three readings, stand at a lower level than the figures in Table IV based on the average of the three readings. According to Du Bois, the lowest two tests which agree closely represent the true basal. Macleod and Rose (1925) selected their lowest observation of basal metabolism as the true basal value. Paul Roth says 'In a series of tests made the same day or within a few days all conditions being equal, the lower basal rates obtained should always be considered as probably the more correct ones. With good technique, it is next to impossible for any subject to develop a rate lower than his true basal rate, while it is obvious, that in any case whether the results are

TABLE III
Summary of basal metabolic rate determinations on 73 young men and women according to ages
(Taking the minimum of three readings as a rule)

Taking the minimum of three readings													
Age Years	MEN						WOMEN						
	Number of cases done	Average basal metabolic rate per sq m per hour Cal	AVERAGE DEVIATION OF ACTUAL FROM PREDICTED PER CENT				Number of cases done	Average basal metabolic rate per sq m per hour Cal	AVERAGE DEVIATION OF ACTUAL FROM PREDICTED PER CENT				
			Aub Du Bois' standard	Harris Benedict's standard	Dreyer's standard	Krogh's standard			Aub Du Bois' standard	Harris Benedict's standard	Dreyer's standard	Krogh's standard	
18	4	39.8	-3.0	0.6	-2.3	1.0	1	31.6	-16.5	-15.1	-16.0	-11.3	-8.5
19	5	34.6	-15.5	-11.9	-13.7	-10.8	1	26.8	-27.5	-30.4	-28.4	-24.3	-26.8
20	8	36.6	-7.6	-7.0	-7.6	-4.2	4	30.7	-16.9	-21.2	-19.0	-16.7	-12.5
21	12	35.1	-11.3	-10.4	-11.6	-8.0	3	31.3	-15.3	-17.0	-17.1	-10.5	-9.2
22	11	34.0	-14.0	-12.3	-13.7	-10.3	5	31.3	-15.7	-15.9	-15.1	-10.5	-8.2
23	13	34.1	-13.7	-12.6	-13.1	-10.0	1	34.2	-7.0	-12.1	-8.8	-1.7	-2.0
24	4	34.9	-11.7	-9.6	-11.5	-6.6							
25	1	36.5	-7.7	-5.2	-9.5	-2.3							
Average	58		-11.6	-10.0	-11.3	-7.6	15		-16.2	-18.2	-17.1	-12.7	
Average for 20-25	49	34.8	-11.8	-10.7	-11.7	-8.2	14	31.0	.				
Average for 19-25	54	34.8	-12.0	-10.8	-11.9	-8.4	15	31.0					-11.0

TABLE IV

Summary of basal metabolic rate determinations on 73 young men and women according to ages
(Taking the average of three readings as a rule)

Age Years	MEN				WOMEN				Difference between men and women per cent	
	Number of cases done	Average basal metabolic rate per sq m per hour Cal	AVERAGE DEVIATION OF ACTUAL FROM PREDICTED PER CENT			Average basal metabolic rate per sq m per hour Cal	AVERAGE DEVIATION OF ACTUAL FROM PREDICTED PER CENT			
			Aub-Du Bois' standard	Harris Benedict's standard	Droyer's standard	Krogh's standard				
18	4	41.3	0.4	4.2	1.0	5.2	-10.7	-6.8	-8.6	-13.6
19	5	36.6	-10.7	-6.8	-8.6	-5.6	-3.4	-2.8	-19.2	-25.0
20	8	38.2	-3.4	-2.8	-3.5		-7.1	-6.0	-14.2	-12.2
21	12	36.8	-7.1	-6.0	-7.4	-3.7	-9.1	-7.1	-11.8	-9.6
22	11	35.9	-9.1	-7.1	-8.6	-5.2	-8.3	-7.0	-13.0	-11.5
23	13	36.3	-8.3	-7.0	-7.6	-4.2	-7.4	-4.9	-8.0	-2.1
24	1	36.6	-7.4	-4.9	-7.5	-2.2				
25	1	37.4	-5.1	-2.8	-7.2					
Average	58		-7.0	-5.2	-6.7	-3.0			-8.3	
Average for 20-25	49	36.7	-7.2	-5.8	-7.0	-3.3			-13.9	-12.0
Average for 10-25	54	36.7							-13.2	-12.0

high or low, there is always an easy chance for the rate observed to be higher than the true basal'

In view of the above observations which are quite true, figures in Table III only will be used for further discussion in this paper and comparison with the Western standards

Basal metabolism in men —The basal metabolic rate recorded for age 18 in men is 39.8C per sq m per hour. This rate stands at a much higher level than the rates recorded for years 19 to 25, deviates only by 3 per cent from Aub-Du Bois' standard and is above the Harris-Benedict, Dreyer and Kiogb's values by 0.6, 2.3 and 1.0 per cent respectively. But as the figure is based on determinations on four subjects only it cannot represent the true rate for the age 18 in this country. The rate would probably be less if a larger number of subjects be used. However, the rate recorded indicates that basal metabolism at the age of 18 stands at a high level and there is a distinct lowering of the basal metabolism after 18.

For age 19, the average basal metabolic rate is found to be 34.6C which is very low when compared with the rate for age 18. But again, as only five subjects of 19 years of age were used, the rate is not established. It is probable that the rate would be higher if a larger number of tests be done.

For age 20, eight subjects were used, and the rate is found to be 36.6C. After the distinct lowering of basal metabolism after the 18th year, it is not probable that there would be much difference in metabolism in the 19th and 20th years of life, and so if an average of the determinations on all subjects (13) of 19 and 20 years of age be taken, the rate would be 35.8C per sq m per hour. This would represent more or less the true basal metabolic rate for 19 and 20 years of age.

For age 21, determinations were made on 12 subjects, for age 22, on 11 subjects, and for age 23 on 13 subjects and the average basal metabolic rates calculated are 35.1C, 34.0C and 34.1C respectively per sq m per hour. These rates may be considered to be more or less established as they are based on determinations on a larger number of cases.

For ages 24 and 25, the subjects were again few and the results cannot be taken to represent the true averages, but as the basal metabolism at these ages will not differ much from the metabolism at 22 and 23 years of age, a probable rate 34.0C may be safely predicted.

Judging from the results for ages 19 to 25, and having noted the basal metabolism at 18 to be at a high level, the rate for the age 18, may also be predicted to be about 37.0C per sq m per hour, which would be 1.2C above the rate for age 19.

So the basal metabolic rates for ages 18 to 25 actually calculated or predicted, and applicable to South India, may be laid down as follows —

Age Years	Du Bois' standard Cal	Krogh's standard Cal	Basal metabolic rate per sq m per hour Cal
18	41 0	39 4	37 0 predicted
19	41 0	38 8	35 8 observed
20	39 5	38 2	35 8 „
21	39 5	38 2	35 8 „
22	39 5	37 9	34 0 „
23	39 5	37 9	34 0 „
24	39 5	37 4	34 0 predicted
25	39 5	37 4	34 0 „
Average	39 8	38 1	34 96
Average deviation from Du Bois' standard			12 1 per cent
„ „ „ Krogh's			8 2 „

The basal metabolic rates could be given in more rounded figures and adjusted as below —

Age Years	Du Bois' standard Cal	Krogh's standard Cal	Basal metabolic rates applicable in South India per sq m per hour Cal
18	41 0	39 4	37 0
19	41 0	38 8	36 0
20	39 5	38 2	35 5
21	39 5	38 2	35 0
22	39 5	37 9	34 5
23	39 5	37 9	34 5
24	39 5	37 4	34 0
25	39 5	37 4	34 0
Average	39 8	38 1	35 0
Average deviation from Du Bois' standard			12 0 per cent
„ „ „ Krogh's			8 1 „

The average for ages 19 to 25 according to the prediction works out to be 34.8 C per sq m per hour, which is the same as the calculated average in Table III, and the average for ages 18 to 25 to be 35.0 C per sq m per hour. This average deviates -12 per cent from Aub-Du Bois, -10.8 per cent from Harris-Benedict, -11.9 per cent from Dreyer and -8.1 per cent from Krogh's modification of Du Bois' standard.

Excluding cases Nos. 8, 13 and 37, the basal metabolism results calculated range from -25.6 per cent to 2.8 per cent (Du Bois), from -24.1 per cent to 2.1 per cent (Harris-Benedict) and from -26.5 per cent to 3.8 per cent (Dreyer). The maximum individual variation from the average basal metabolic rates for 19 to 25 years is found to be 16.6 per cent or -15.5 per cent (Table V).

TABLE V

Summary of basal metabolic rate determinations on 73 young men and women showing maximum deviation from average

(Taking the minimum of three readings as a rule)

Age Years	MEN			WOMEN		
	Number of cases done	Basal metabolic rates per hour per sq m Cal	Maximum deviation from average per cent	Number of cases done	Basal metabolic rates per hour per sq m Cal	Maximum deviation from average per cent
18	4	39.8	5.5 or -4.0			
19	5	34.6	13.9 „ -9.5	1	31.6	
20	8	36.6	9.0 „ -13.9	1	26.8	
21	12	35.1	10.0 „ -12.2	4	30.7	10.7 or -7.2
22	11	34.0	12.6 „ -13.2	3	31.3	1.1 „ -4.8
23	13	34.1	19.0 „ -12.0	5	31.3	13.7 „ -5.7
24	4	34.9	11.7 „ -15.7	1	34.2	
25	1	36.5				
20-25	49	34.8	16.6 or -15.5	14	31.0	14.8 or -13.5
19-25	54	34.8	16.6 „ -15.5	15	31.0	14.8 „ -13.5

Of all the average deviations, those from Krogh's standard (reduced Du Bois' standard) are the lowest and those from Aub-Du Bois and Dreyer's are the highest and practically similar

Since the Aub-Du Bois' values, though high, are more in common use and have been used invariably for comparison by workers on metabolism in the West, I need refer only to the deviations from Du Bois in further discussion. Moreover as August Krogh observed, Harris-Benedict tables are less reliable for persons of exceptional build. This is well illustrated in one of my cases, No 57, who was rather obese, weighing 80.9 kilos and had a body surface of 1.96 sq. m. In this case the deviation was 2.8 per cent above Du Bois but only 0.9 per cent above Harris-Benedict. Again in cases Nos 4, 8, 25, 43, 55 and 59, Harris-Benedict deviations are practically the same as or a little more than Du Bois, though in average subjects the Harris-Benedict values are lower than Du Bois or Dreyer's values.

Basal metabolism in women — Only fifteen women students (19 to 24 years) were available, one each of 19, 20 and 24 years of age and 3 to 5 cases each of 21, 22 and 23 years of age. The average basal metabolic rate for ages 21 to 23 (12 subjects in all) is calculated to be 31.10 C per sq. m. per hour and this may be taken as the probable average for those years. The general average calculated for all the fifteen subjects is 31.00 C (Table III). In view of the low metabolic rates recorded for all the ages except in case of age 24, the rates for years 19 to 25 may be laid down as follows —

Age Years	Basal metabolic rate predicted Cal	Krogh's standard Cal	Du Bois' standard Cal
19	32.0	35.9	38.0
20	31.5	35.4	37.0
21	31.5	35.4	37.0
22	31.0	35.0	37.0
23	31.0	35.0	37.0
24	30.5	34.8	37.0
25	30.5	34.8	37.0

This would give an average of 31.20 C showing a difference of 10 per cent from the figures for men in South India, and a deviation of —16.0 per cent from Du Bois, —18.0 per cent from Harris-Benedict, —17.0 per cent from Dreyer and —12.5 per cent from Krogh's standards.

In Charts 1 and 2 the level of basal metabolism and the variation according to age of the predicted values for men and women in South India, as compared with

CHART I

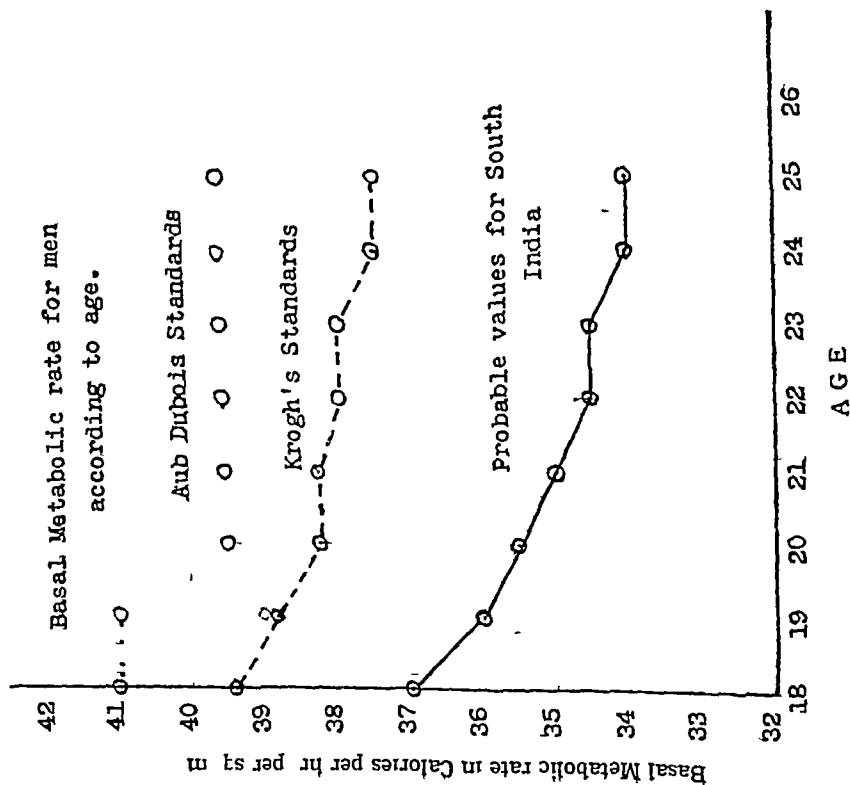
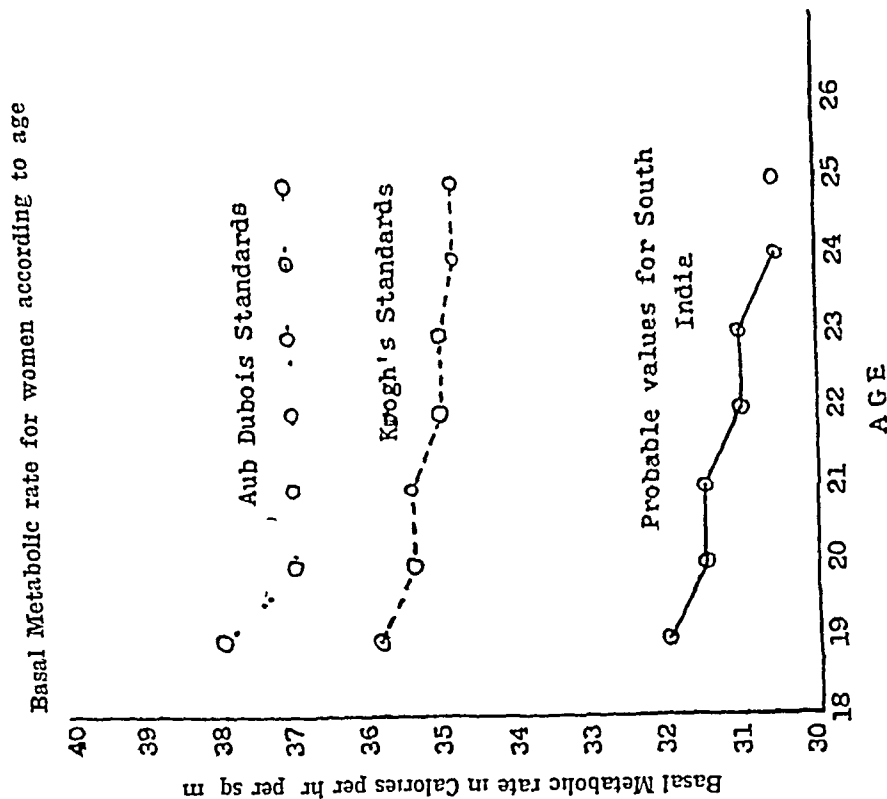


CHART 2



the Aub-Du Bois and Krogh's values are graphically represented. Charts 3 and 4 show graphically the variation of basal metabolism according to weight and body surface in South India as compared with Aub-Du Bois and Krogh's variations. No correlation between pulse rate and basal metabolism was found.

Discussion—The deviations from Du Bois' values for men and women have been found to be —12 per cent and —16 per cent.

In 1923, August Krogh found the Du Bois' results on an average too high and in 1925 published Du Bois' tables with a uniform 6 per cent reduction.

According to E. F. Du Bois (1927), Aub and Du Bois' standards of 1917 are 6 per cent too high. F. G. Benedict believes that the present standards for American women are approximately 5 per cent too high.

Blunt and Dye (1921) studied 17 women between 24 and 44 years and found the figures averaged 4.1 per cent below the Harris-Benedict's and 6.5 per cent below the Aub-Du Bois' standards. Macleod and Rose studied 92 normal women between the ages of 20 and 50 years and the figures averaged 4.4 per cent below the Harris-Benedict's and 8.6 per cent below the Aub-Du Bois' values.

So, on an average, if the Du Bois' values are considered to be 6 per cent too high for Americans themselves we have still to account for —6 per cent and —10 per cent deviation in case of men and women respectively of South India.

The question whether the variation of basal metabolism in different parts of the world is due to racial differences has been investigated by several workers. Benedict and his collaborators showed that the basal metabolic rate of a group of Oriental students living for a considerable time in Chicago under absolutely Western conditions was distinctly below Western standards. Macleod, Crofts and Benedict (1925) studied 7 Chinese and 2 Japanese women students who were in New York climate for 15 months. The basal metabolism was 10.4 per cent below Harris-Benedict and 10.2 per cent below the Sage standards. But as the standards were 5 per cent too high for American women themselves, the Orientals were still 5 per cent below. But Eijkmann (1896) working in far off Batavia could find no significant change in the basal metabolism of Malayan servants and the Europeans living in Batavia. A. Ozario de Almeida (1920) working in Brazil found the basal metabolism of the white men averaged 24 per cent below in one series and in another 16.2 per cent below the American standards and that of the Negro labourers was 8 per cent higher than the Whites but still below the figures in temperate zone. According to him, the basal metabolism depends not on race but on muscular work, level of food intake and the climate.

Knipping and Fleming (1923) working independently found the basal metabolism of Europeans, Malays and Chinese living for a long time in the tropics averaged 6.3 and 8.3 per cent below the Benedict's standards. Fleming also noted in the Philippines that one American, whose basal metabolism was 3 per cent below the

CHART 4

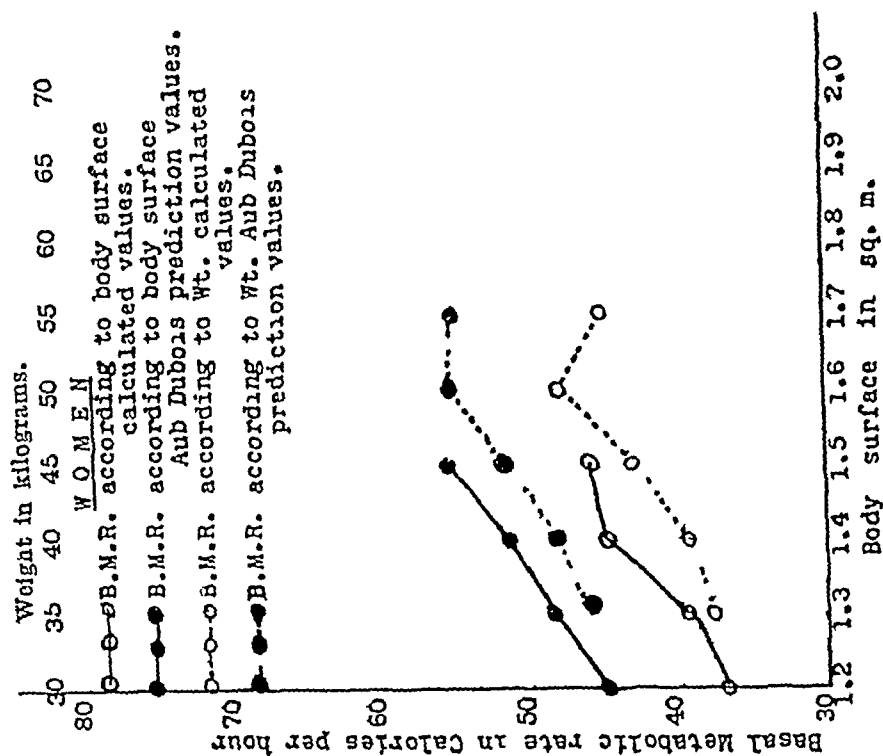
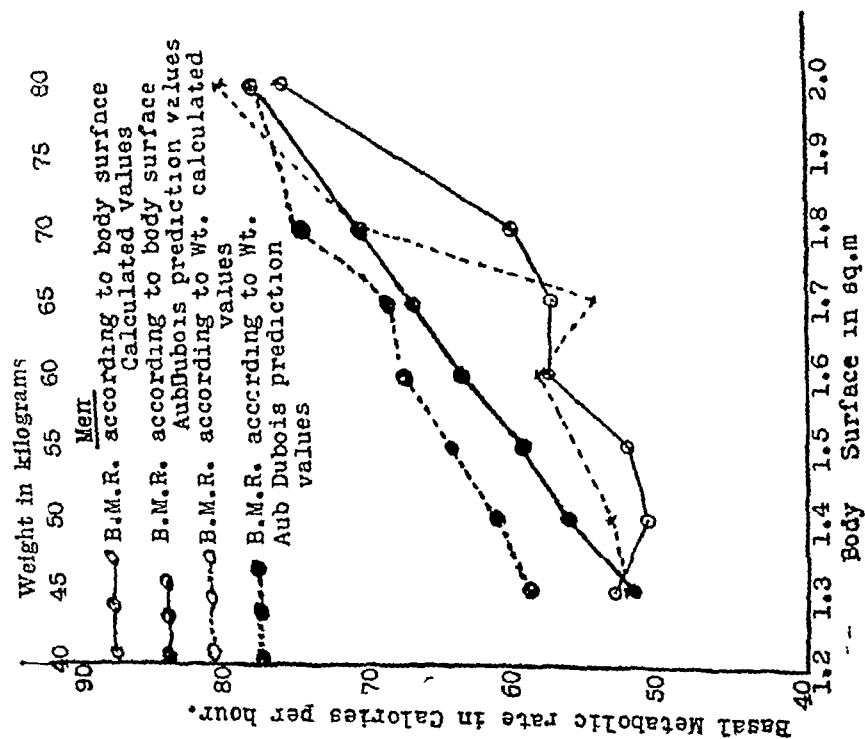


CHART 3.



standard, showed, on arrival in the Philippines, a fall to —7 per cent after 13 months' residence in the islands

Takahara (1925) studied the metabolism of 120 Japanese men and women and reported that the average for men was 5.5 per cent below the Sage (Aub-Du Bois) standards while the women were 7.3 per cent below. He did not believe that there was any significant difference between the two different races and all workers have to agree with him if 6 per cent reduction in the Aub-Du Bois standard be accepted. The lowered metabolism in the Oriental students living in America, noted by Benedict, Macleod and Crofts, must be due not to racial difference but to other causes which will be discussed below.

If the racial factor is eliminated, the lowered metabolism must be due to either climatic conditions or influence of diet or the general physique of the individuals.

Leonard Hill and others (1922) found the metabolism of children was 20 to 30 per cent higher in winter than in summer. E. Hindmarsh (1927), working in Sydney, found an average reduction of 9.6 per cent for the men and 10.9 per cent for the women from Du Bois' predicted values. He suggests that this reduced basal metabolic rate is due to the more ready relaxation of the subjects in the warmer climate of Sydney. M. Heggerda and Benedict (1928) found male brown individuals in Jamaica had an average of 5.1 per cent below the American White average.

H. Neeches (1930) observed, during a prolonged stay in China, that the average Chinese is always more relaxed than the average Westerner. Their muscular tone seemed to be constantly lower than that of Westerners, which, he conjectured might be the reason for their lower basal metabolic rates. He confirmed the hypothesis by finding very little difference in their basal metabolic rate between waking and sleeping. With Westerners, there was considerable drop during sleep, indicating a high muscular tone and so a high basal metabolism in the waking state.

So there is evidence that the low basal metabolic rate of Orientals is at any rate partly due to a greater degree of constant relaxation. This relaxation is apparently due to the influence of the warm climate. If the climate of Sydney is sufficient to produce ready relaxation of the subjects as found by E. Hindmarsh, how much more responsible should the hot tropical climate of Madras be to produce relaxation of the subjects and lower their basal metabolism. Climate may not affect metabolism directly but does indirectly, by the virtue of habits and activities acquired.

Another factor, which, I believe, plays a more important part in the maintenance of a high or low basal metabolism, is the influence of diet. Under-nutrition is an important factor in the reduction of basal metabolism. Krogh and Lindhard (1920-22) believe that the metabolism is distinctly lower if the previous diet has been low in protein. The average diet in South India, whether vegetarian or non-vegetarian, is deficient in protein and fat, the staple food being rice. The

so-called non-vegetarians here, with a few exceptions, take very little meat, and ordinarily they have meat or fish or egg in a curried form three or four times a week or utmost once a day along with rice and vegetables

The standards of basal metabolism in the West were devised for well-nourished people in good health and who take mixed diet. The larger the protein content in food, the higher is the basal metabolism (Lusk). It seems fairly conclusively proved that high calories and especially the quantity of protein taken has decided positive influence on basal metabolism. Kleitman has conclusively shown this by altering the previous daily diet and estimating the basal calories. In my series of cases, I have compared the basal metabolism of a few (7 cases) who were definitely known to be on a high protein diet and high calories (Table VI) with that of

TABLE VI

Basal metabolic rates observed in persons who were known to be on a high protein diet

Number	Number of subject	Age Years	Weight in kilo	Sitting height cm	Body surface sq m	Caste	Diet	Basal metabolic rate per sq m per hour Cal	Calculated average for the age Cal	Aub Du Bois' standard Cal	Predicted value for the age Cal
1	8	20	56.0	84	1.60	N Br	N Veg	41.1	36.6	39.5	35.5
2	27	20	52.7	86	1.64	A I	N Veg	39.9	36.6	39.5	35.5
3	59	21	69.5	98	1.81	Br	Veg	38.2	35.1	39.5	35.0
4	23	22	54.8	85	1.63	N Br	Veg	38.3	34.0	39.5	34.5
5	4	23	62.0	87	1.65	Br	Veg	40.2	34.1	39.5	34.5
6	37	23	52.0	87	1.53	N Br	N Veg	41.6	34.1	39.5	34.5
7	57	23	80.9	92	1.96	Br	Veg	40.6	34.1	39.5	34.5
Average			61.1	87	1.69			40.0	34.8	39.5	34.8

an equal number practically of about the same weight and body surface, who were definitely known to be on a low protein diet (Table VII). The average basal metabolic rate of cases on high protein diet was 40.0 per sq m per hour, while that of cases on low protein diet was only 31.9C, the average calculated or predicted for the whole series being 34.8C.

TABLE VII

Basal metabolic rates observed in persons who were known to be on a low protein diet

Number	Number of subjects	Age Years	Weight in kilo	Sitting height cm	Body surface sq m	Caste	Diet	Basal metabolic rate per sq m per hour Cal	Calculated average for the age Cal	Aub Du Bois' standard Cal	Predicted value for the age Cal
1	30	20	54.0	88	1.63	N Br	Veg	34.7	36.6	39.5	35.5
2	45	21	53.1	89	1.61	N Br	Veg	32.5	35.1	39.5	35.0
3	51	22	50.8	94	1.66	Br	Veg	32.3	34.0	39.5	34.5
4	60	22	57.7	90	1.73	N Br	Veg	29.5	34.0	39.5	34.5
5	39	23	65.0	88	1.78	N Br	N Veg	32.8	34.1	39.5	34.5
6	7	21	51.5	84	1.59	N Br	N Veg	32.1	34.9	39.5	34.0
7	9	24	52.0	84	1.30	N Br	N Veg	29.4	34.9	39.5	34.0
Average			55.7	85	1.65			31.9	34.8	39.5	34.6

So, accepting that the Du Bois' values are 6 per cent too high, a further reduction of 6 per cent in South India can be said to be due to the ready muscular relaxation in a hot tropical climate and a low protein diet.

Influence of exercise—It is said that athletic training pushes up the basal metabolic rate. Benedict and Smith found basal metabolism 6 to 7 per cent higher in athletes. Du Bois found 2.5 per cent higher than the Sage standards. Takahira also found higher basal metabolic rates in general labourers than in teachers and traders.

But in Florida, J. Titt (1930) found the average basal metabolism of ten women students engaged in athletics was the same as that of the whole mixed group. A. H. Heinhaus (1928) in his studies on the influence of exercise on basal metabolism in dogs, found no change as result of increased musculature in adult animals due to exercise. He observes that it is possible to train a sedentary animal to take strenuous exercise without after effects. A period of training sufficient to do this seems to have no effect on the basal metabolism either during the training period or afterwards, though there is a possibility that if very prolonged the basal metabolism may be lowered.

In Table VIII, I have shown the basal metabolic rates of a few of my subjects who were daily indulging in good exercise. Except in the first case, in all others

the basal metabolism was practically the same as, or below, the predicted or calculated values for the ages. The average for the six cases recorded is distinctly lower than the calculated or predicted average for the mixed group.

TABLE VIII

Basal metabolic rates observed in men who were daily indulging in fairly severe exercise

Number	Number of subject	Age Years	Weight in kilo	Sitting height cm	Body surface sq m	Caste	Diet	Basal metabolic rate per sq m per hour Cal	Calculated average for the age Cal	Aub Du Bois' standard Cal	Predicted value for the age Cal
1	25	21	72.0	98	1.95	A I	N Veg	36.6	35.1	39.5	35.0
2	34	21	54.0	83	1.58	I C	N Veg	35.6	35.1	39.5	35.0
3	24	23	61.0	93	1.73	A I	N Veg	30.0	34.1	39.5	34.5
4	46	23	57.2	81	1.62	Moh	N Veg	34.4	34.1	39.5	34.5
5	55	23	63.2	87	1.68	Br	Veg	30.1	34.1	39.5	34.5
6	56	23	61.4	85	1.75	Br	Veg	30.0	34.1	39.5	34.5
Average								32.8	34.8	39.5	34.7

In women also, a further reduction of 10 per cent can be explained as due to a low protein diet and the more marked muscular relaxation in the hot climate. As all the subjects were medical students, they had frequent exposure to open air and also indulged in some sort of exercise, walking or playing badminton or tennis, unlike most of their sisters in the middle and upper classes who hardly take any exercise and lead mostly an indoor life.

The low rates found by Macleod, Crofts and Benedict (1925) in Oriental women students resident in New York for fifteen months, compared with the rates in American women, could be explained by the fact that the Oriental students continued to have the same or a less degree of muscular relaxation they used to have in their country and probably also by the fact, that they were not having the same quantity of food as their American companions had, as they were new to the diet.

Du Bois (1917) calculated the figures for females as 7 per cent below the average for males. Stoner (1924), using Dreyer formula, subtracted 10 per cent for the females. E. F. Du Bois says that sex difference is probably greater in Orientals.

Takahira in Japan found the basal metabolism in women averaged 9 per cent lower than that of the men, according to surface area. In my series, the average basal metabolic rate for women is found to be 10 per cent below the average for men.

Influence of menstruation—Undoubtedly, the menstrual cycle has a profound disturbing effect on metabolism in women. F. G. Benedict and Finn (1928) found oxygen consumption lowest during the menstrual period and highest one week after menstruation. Menstruation is therefore a real factor in lowering metabolism. R. F. Mattern in Australia (1929), from a study of two normal female subjects, found there was a rise in standard metabolism immediately before menstruation and a fall during menstruation.

In Table IX the basal metabolic rates in relation to the menstrual cycle are shown. In my series, no determinations were made during menstruation. In one case aged 21, two days after menstruation, the basal metabolic rate was only

TABLE IX

Basal metabolic rate of normal young women showing its relation to the menstrual cycle

Number of subject.	Age Years	Standing height cm	Weight in kilo	Body surface sq m	Day after last menstruation	Basal metabolic rate per sq m per hour Cal
65	19	159	51.8	1.51	10	31.6
66	20	153	40.5	1.33	18	26.8
62	21	154	46.4	1.41	22	28.5
64	21	150	43.7	1.35	11	34.0
67	21	140	37.3	1.20	7	30.5
68	21	161	37.3	1.34	2	29.8
63	22	150	53.2	1.47	10	29.8
69	22	150	46.4	1.38	11	31.5
76	22	156	50.9	1.49	6	32.6
70	23	150	35.0	1.23	18	30.2
72	23	150	49.1	1.42	15	35.6
73	23	151	56.4	1.51	22	31.0
74	23	162	50.0	1.52	9	30.0
71	23	163	46.4	1.47	13	29.5
75	24	150	38.7	1.27	26	34.2

29.8°C the normal average calculated for the age being 30.7°C and the predicted value being 31.5°C per sq m per hour, showing thereby that basal metabolism continues to be low for a few days after menstruation. Subject No. 75 aged 24 on the 26th day after menstruation had a high rate of 34.2°C when the predicted value is 30.5 and the total average is 31.2. This shows a rise in basal metabolism in the pre-menstrual period.

It may be gathered from the figures in Table IX, that on the average, though there is no uniformity, there is a rise during the second week and a decline during the third week after menstruation. There is a pre-menstrual rise again and then a fall during the menstruation which continues probably for a week after.

TABLE X

Du Bois' body surface formula and Meeh's body surface formula compared

Number of subject	Body surface Du Bois' formula sq m	Body surface Meeh's formula sq m	Basal metabolic rate per sq m per hour using Du Bois' formula Cal	Basal metabolic rate per sq m per hour using Meeh's formula Cal
b	1.59	1.73	38.2	35.0
17	1.27	1.35	42.0	39.5
22	1.49	1.63	31.4	28.7
27	1.64	1.73	39.9	32.9
43	1.70	1.89	36.5	33.0
25	1.95	2.13	36.6	33.4
45	1.61	1.74	32.5	30.1
60	1.73	1.84	29.5	27.7
57	1.96	2.30	40.6	34.6
65	1.51	1.71	31.6	28.0
67	1.20	1.37	30.5	26.8
74	1.52	1.67	30.0	27.2

SUMMARY

With a view to find normal standards of basal metabolism for men and women in South India, data were collected on 76 medical college students in Madras, of whom 15 were women, of ages varying from 18 years to 25 years

The subjects were brought to the Laboratory by car under basal conditions, i.e., 14 to 18 hours after the last meal and without breakfast in the morning or were kept at a convenient place overnight with an early evening meal. The basal metabolism was measured in the morning before breakfast after 30 to 45 minutes' complete rest. The determinations were made on each case on two different days. The apparatus used was the latest model of Benedict-Roth, with rubber flutter valves and the Collin's Kymograph attachment.

The body surface was computed from the charts devised by Aub and Du Bois based on height-weight formula.

About 230 basal metabolism determinations were made in all. As advocated by Du Bois, Macleod, Rose and Paul Roth, only the minimal basal rates were taken into consideration and compared with the standards of Du Bois, Harris-Benedict, Dreyer and Krogh. The data collected and the deviations from standards are given in Tables I and II. The results are summarized according to ages in Tables III and IV.

In men, the basal metabolic rate calculated for age 18 was found to be much higher than the average for ages 19 to 25, but being based on determinations on only four subjects the figure is not considered to represent the true rate for 18 years, though it is an indication of the high level of metabolism at that age.

For ages 19 to 25 the average basal metabolic rate is found to be 34.80 per sq. m. per hour and may be considered fairly well established. Judging from the results, the probable values applicable to South India for ages 18 to 25 are laid down as follows: 37.00 for age 18, 36.0 for 19, 35.5 for 20, 35.0 for 21, 34.5 for ages 22 and 23, and 34.0 for 24 and 25.

The average 35.00 deviates —12 per cent from Du Bois, —10.8 per cent from Harris-Benedict, —11.9 per cent from Dreyer and —8.1 per cent from Krogh's standards. The basal metabolism results range from —25.6 per cent to 2.8 per cent (Du Bois), from —24.1 to 2.1 per cent (Harris-Benedict), and from —26.5 per cent to 3.8 per cent (Dreyer). The maximum individual variation from the average basal metabolic rate is found to be 16.6 per cent or —15.5 per cent.

In women (19 to 24 years of age) the predicted values are laid down as 32.00 for age 19, 31.50 for ages 20 and 21, 31.00 for 22 and 23 and 30.50 for 24 and 25 which gives an average of 31.00 per sq. m. per hour, same as the figure actually calculated from the results. This average shows a difference of 10 per cent from

the rate for men, and deviates —16 per cent from Du Bois, —18 per cent from Harris-Benedict, —17 per cent from Dreyer, and —12.5 per cent from Krogh's standards

The level of basal metabolism in South India and the variations according to age, weight and body surface as compared with the Du Bois' and Krogh's standards are represented graphically in Charts 1 to 4. No correlation between pulse rate and metabolism was found.

The factors which account for the lowering of basal metabolism in South India by 12 per cent in men and 16 per cent in women as compared with the Du Bois' standards are discussed. The findings of August Krogh and E. F. Du Bois are quoted to show that the Aub-Du Bois' rates are 6 per cent too high even for Westerners. The findings of other workers on metabolism like Ovario de Almeida in Brazil, Takahira in Japan, etc., are quoted to show that lowering of metabolism cannot be due to any racial difference. The view is put forward that the lowering is due to the ready muscular relaxation in the hot tropical climate of Madras and to the low protein diet in South India, where the average diet of a vegetarian or a non-vegetarian is deficient in protein and fat, the staple food being rice. The so-called non-vegetarians in South India, with a few exceptions, take very little meat.

The basal metabolism of a few cases on a high protein diet, selected from the series investigated, is compared with that of a few others on a low protein diet (Tables VI and VII). The average basal metabolic rate in the former is found to be 40.0 C and in the latter only 31.9 C per sq. m. per hour.

To show the effects of exercise on basal metabolism a few subjects known to indulge daily in exercise were selected and their basal metabolism rates are shown in Table VIII. The average basal metabolic rate is found to be distinctly lower than the calculated or predicted average for the mixed group.

In women the low metabolism is again attributed to the more marked muscular relaxation and the low protein diet obtainable here. The influence of the menstrual cycle on basal metabolism is commented on, and the rates in relation to the menstrual cycle are shown in Table IX. A rise in basal metabolism is noted in the pre-menstrual period and in the second week after menstruation. At other periods of the cycle there is a decline.

I wish to acknowledge my indebtedness to the students, men and women, who so readily acted as subjects and to Dr. Thomas, warden of the women-students hostel, for her kind co-operation and help in collecting the data on women subjects.

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Since sending this article to the Press, Miss E D Mason from the Women's Christian College, Madras, and P G Benedict from the Nutrition Laboratory, Carnegie Institute, Boston, U S A, have published in the *Indian Journal of Medical Research* **19**, 1, July, 1931, the results of their observations on the basal metabolism of 51 South Indian women of 17 to 31 years of age. They find the basal metabolism to average 17.2 per cent below the Du Bois' standards.

STUDIES IN THE NUTRITIVE VALUE OF INDIAN VEGETABLE FOOD-STUFFS

Part III.

NUTRITIVE VALUES OF LENTIL, *LENS ESCULENTA* MOENCH,
COW PEA, *VIGNA CATJANG*, WALP AND ACONITE
BEAN, *PHASEOLUS ACONITIFOLIUS*, JACQ

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[Received for publication, August 7, 1931]

THE lentil, *Lens esculenta*, is a valuable pulse grown as a winter crop all over India. It is cultivated extensively in the United and Central Provinces, where it is used as a daily food in place of tuar dal, *Cajanus indicus*. In India it is eaten as 'dal' flavoured with various aromatics and condiments and is considered to be highly nutritious. In some places the young pod is also eaten as a vegetable and the dry leaves and stalks are generally priced as fodder. In Europe the pulse meal mixed with barley flour or other cereal is used as invalid food. The vernacular names are Masur, Channangi, Mohr, Misurpappu, etc.

The cow pea, *Vigna catjang*, is a suberect herb cultivated in the hotter parts of India. The crop, as a rule, is grown for its seeds and used as a pulse. It may be cultivated alone, but is usually a subordinate crop. Various races exist, one of which, with long pods is raised as a vegetable and sold as a substitute for French beans. The vernacular names are Chowlee, Lobia, Barbatti, Caramunny, Bobberlu, Tadagunny, etc.

The aconite bean or kidney bean, *Phaseolus aconitifolius*, is a perennial or annual herb found throughout India and cultivated as a hot-weather crop and

reaped in the autumn. In the United Provinces and Bombay it forms an important crop. The pulse is split and eaten as dal and also cooked in various ways. The most common vernacular names are Math, Matgi, Birmung, Tulkpyre, Kunkuma pesalu, etc.

This part deals with the isolation and analysis of the total globulins of the above three pulses and the estimation of the biological value of the total proteins in their seeds.

The seed coat of the lentil was removed before grinding, while the other two pulses—cow pea and aconite bean—were ground whole as they are usually eaten as such. The pulse flours were all passed through a 40-mesh sieve and they gave the following percentage on analysis —

TABLE I

Pulse	Ash	Ether extractives	Crude fibre	Crude protein N \times 6.25	Carbohydrates (by difference)	True protein (determined separately)
<i>Lens esculenta</i>	3.45	1.75	1.15	23.74	64.93	27.40
<i>Vigna catjang</i>	3.90	1.24	4.38	26.02	64.37	24.74
<i>Phaseolus aconitifolius</i>	4.05	0.32	4.63	27.09	63.91	24.81

The methods of isolation and analysis of the globulins and of the determination of the biological values have been discussed fully in Part I of this series (1931) and only special features need mention here.

The saline extract of *Lens esculenta* removed a good deal of the pink colouring matter from the pulse and this was partly adsorbed by the globulin on precipitation. Even after repeated washings, the pink colour could not be removed completely, so that the globulin of this pulse was faintly pinkish in colour. The globulins of *Phaseolus aconitifolius* and *Vigna catjang* were respectively greyish and white in colour. Elementary analysis of the preparations gave the following percentages.

TABLE II

	<i>Lens esculenta</i>		<i>Vigna catjang</i>		<i>Phaseolus aconitifolius</i>	
Moisture	11.26	11.77	7.74	7.54	9.84	9.96
Ash	0.72	0.73	0.52	0.41	0.73	0.63
On ash and moisture free basis						
Nitrogen	16.17	16.50	15.85	15.28	15.93	16.04
Sulphur	0.423	0.44	0.538	0.526	0.452	0.414

The results of the Van Slyke analyses of these preparations and of the separate determinations of the essential amino-acids in them, are given in Tables III and IV

TABLE III

Analyses of the globulins by the Van Slyke method

(Expressed as per cent of total nitrogen)

Form of nitrogen	Globulin of <i>Lens esculenta</i>		Globulin of <i>Phaseolus acontifolius</i>		Globulin of <i>Ligna calyana</i>	
	1	2	1	2	1	2
Acid insoluble malanin	0.49	0.45	0.82	0.93	0.94	0.85
Acid soluble malanin (adsorbed by lime)	0.65	0.67	0.86	0.78	0.87	0.83
Amide	10.50	9.91	11.89	12.14	10.86	11.40
Diamino —						
Arginine	20.81	19.99	15.92	15.53	15.42	15.03
Histidine	4.93	4.24	5.03	5.64	3.98	3.61
Cystine	0.48	0.51	0.42	0.38	0.56	0.48
Lysine	7.59	8.13	6.96	6.21	8.52	9.75
Mono amino —						
Amino	53.53	54.11	56.02	55.24	51.62	53.93
Non amino	2.09	2.62	2.98	3.38	3.99	4.25
TOTAL	101.07	100.63	100.90	100.23	99.76	100.13

Free amino nitrogen in the native proteins

By direct estimation	4.11	3.61	4.92
Half lysine nitrogen	3.93	3.29	4.57

TABLE IV

Expressed as per cent of protein (ash- and moisture-free)

Amino acid	Globulin of <i>Lens esculenta</i>	Globulin of <i>Phaseolus acontifolius</i>	Globulin of <i>Vigna catjang</i>	Method
Lysine	6.70	5.40	5.96	Van Slyke
Histidine	2.77	3.15	2.21	Van Slyke
Arginine	10.35	7.81	7.45	Van Slyke
Arginine	11.53	8.62	7.91	Plimmer and Rosedale (1925)
Cystine	1.62	1.62	1.89	Remington (1930)
Tyrosine	3.56	3.49	3.74	Folin and Marenzio (1929)
Tyrosine	3.92	3.76	3.88	Zawerski (1926)
Tryptophane	0.71	0.67	0.80	Folin and Marenzio (1929)
Tryptophane	0.62	0.74	0.59	Tillman and Alt (1925)

The metabolic experiments were carried out in a new type of cage. This is a modified form of the Coonoor type of cage, the use of which was kindly suggested to us by Col. McCarrison of the Coonoor Institute. The accompanying photograph and sketch (Plate XLVI, figs. 1 and 2) give an idea about the construction of these cages. The cylindrical body of the cage is made of fine mesh wire gauze. The lid, side tube, inner tube, funnel and the contrivance for holding the glass water tube are all made of galvanized iron. As the funnel is lined with paraffin, the urine does not stick to its sides which can be daily washed down, with dilute sulphuric acid. The inner tube is of a size just sufficient for the rats to pass through, but not spacious enough to allow them to turn back. As it is removable, it can be replaced by one of the desired size for different sizes of rats. The animal gets in through the inner tube and eats the food by projecting its mouth into the food container, and thus is prevented from scattering the food.

The rations contained 10 per cent of protein and were prepared in the same way as given in the previous paper. The metabolism data are given in Table V.

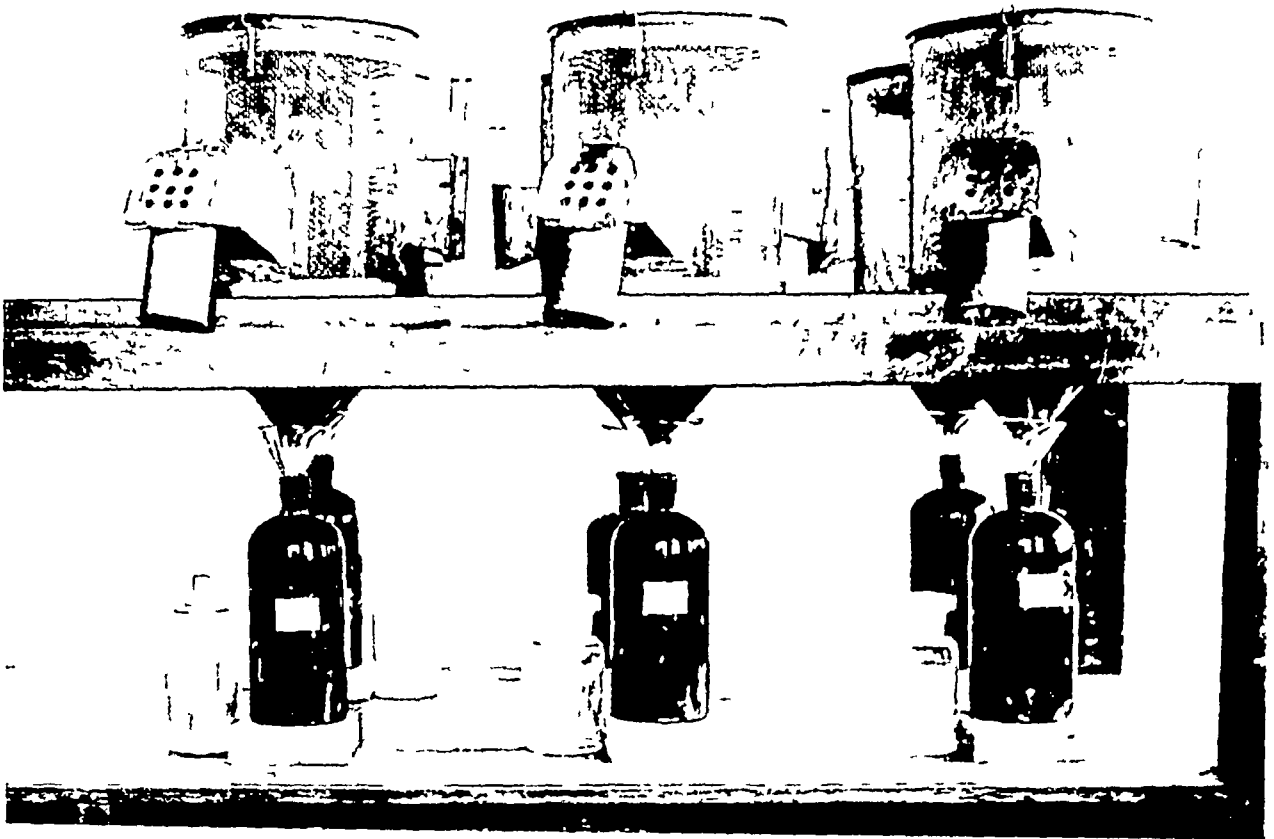
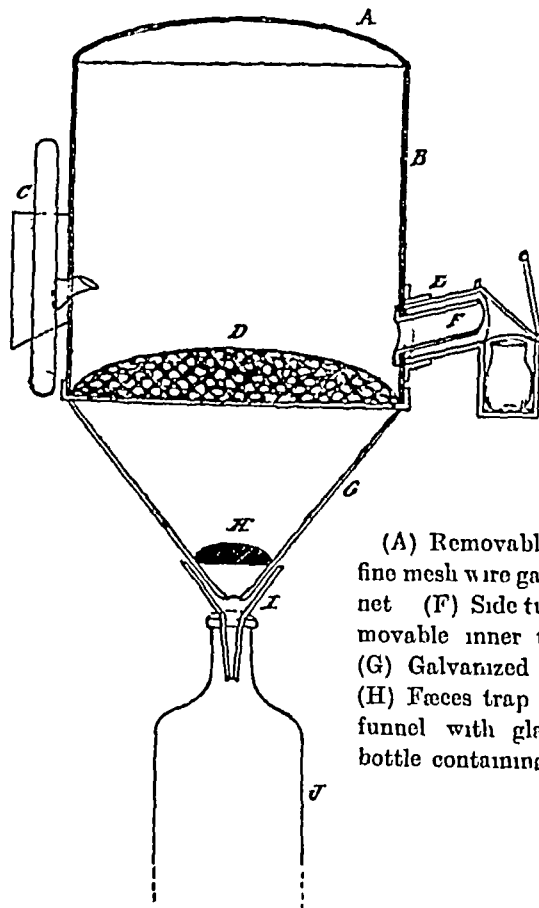


Fig 1



(A) Removable lid (B) Cylindrical cage of fine mesh wire gauze (C) Water tube (D) Wire net (E) Side tube with food container (F) Removable inner tube sliding into the side tube (G) Galvanized iron funnel lined with paraffin (H) Faeces trap of fine wire mesh (I) Glass funnel with glass wool (J) Urine collecting bottle containing dilute sulphuric acid.

Fig. 2.

TABLE V—*concd*

Number	Initial weight	Final weight	Food intake	Nitrogen intake	Faecal nitrogen	Urinary nitrogen	Metabolic nitrogen per gram of food	Endogenous nitrogen per 100 gram body-weight	Food nitrogen in faeces	Absorbed nitrogen	Food nitrogen in urine	Food nitrogen retained	Digestibility	Biological value
	g	g	g	mg	mg	mg	mg	mg	mg	mg	mg	mg	Per cent	Per cent
<i>Period 2 Lens esculenta ration (N = 1.770 per cent)</i>														
19	85.0	85.0	5.11	90.44	37.58	49.34			21.74	68.70	30.50	38.20	76	56
20	60.5	62.5	3.89	68.85	28.81	34.40			16.15	52.70	23.26	29.44	77	56
21	62.9	64.0	3.59	63.54	27.13	34.64			14.44	49.10	19.64	29.46	77	60
22	56.0	60.0	3.76	66.56	26.89	34.46			14.64	51.92	20.69	31.23	78	60
23	62.2	65.8	4.11	72.74	27.26	38.90			15.42	57.32	24.12	33.20	79	58
24	73.1	77.8	4.51	79.83	28.93	42.12			15.89	63.94	26.09	37.85	80	59
											Average		78	59
<i>Period 3 Phaseolus aconitifolius ration (N = 1.923 per cent)</i>														
19	84.4	80.0	5.57	101.60	72.51	40.56			53.76	57.15	24.05	33.10	56	58
20	62.0	57.8	4.30	78.39	49.87	30.26			34.33	44.06	19.47	24.49	56	56
21	62.0	59.0	3.73	68.00	42.64	30.21			28.22	39.78	16.31	23.47	59	59
22	57.0	55.0	4.37	79.68	48.74	33.50			32.24	47.44	21.15	26.20	60	56
23	62.8	60.1	4.90	89.33	48.74	34.20			33.93	55.40	21.90	33.49	62	60
24	75.0	72.6	5.12	93.14	55.51	38.85			39.49	53.65	24.42	29.23	58	55
											Average		58.5	57

J, MR	Period 4 Vigna catjang ration ($N = 1.676$ per cent)									
	19	20	21	22	23	24	12.29	15.81	30.79	73
	82.2	58.0	60.1	55.5	62.5	73.9	7.79	36.32	22.01	79
	81.0	58.0	58.0	55.3	62.8	75.1	8.96	30.76	21.20	71
	4.57	3.48	3.10	3.55	4.20	4.10	10.95	34.75	19.18	69
	76.60	58.33	51.96	59.51	70.39	68.72	11.14	38.93	31.16	71
	47.41	35.85	34.22	34.43	44.74	44.58	10.20	37.93	30.79	72
	26.00	18.19	21.97	22.21	23.76	23.54	Average			
										58

Non protein ration ($N = 0.842$ per cent)												
Per od 5		81.0	74.8	2.83	2.38	11.05	12.43	3.902	15.97			
19												
20		57.1	53.1	1.80	1.32	7.80	9.83	4.735	17.84			
21		56.1	52.7	1.67	1.40	7.56	11.89	4.529	21.85			
22		55.7	51.5	1.87	1.57	9.00	10.01	4.815	18.67			
23		62.0	57.0	2.40	2.02	7.92	11.09	3.701	18.65			
24		74.0	69.6	2.70	2.27	9.72	11.67	3.601	16.25			

The analysis of the three globulins shows that the globulin of *Lens esculenta* is characterized by its higher content of lysine and arginine. The net protein values $\frac{\text{Content of digestible protein} \times \text{biological value}}{100}$ of the pulse calculated from the metabolism data are given in the following table —

TABLE VI

Pulse	Total protein N \times 6.25 Per cent	Net protein value Per cent
<i>Lens esculenta</i>	28.74	12.86
<i>Vigna catjang</i>	26.52	11.05
<i>Phaseolus aconitifolius</i>	27.09	9.04

At a 10 per cent level of intake, the proteins of *Lens esculenta* are highly digestible, while those of *Vigna catjang* have a high biological value. *Phaseolus aconitifolius* is poor in both respects.

The pulses previously investigated at the same level of protein intake are *Dolichos biflorus*, *Cicer arietinum* and *Dolichos lablab*, and their net protein values are 10.43, 16.68 and 10.65 per cent respectively.

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BACTERIOLOGICAL EXAMINATION IN LEPROSY.

A STUDY IN THE EFFICIENCY OF THE VARIOUS METHODS IN COMMON USE

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[Received for publication, August 10, 1931]

INTRODUCTORY

THE *Mycobacterium lepræ* was first discovered by Hansen in sections of the nodules of lepers. Since then many workers have found the *M lepræ* constantly present in most of the skin lesions of leprosy. Various workers have advocated various techniques for demonstrating the organism. In 1897 Sticker reported the occurrence of *M lepræ* in the nasal discharge of 128 out of 153 patients examined and concluded that the nasal lesion is the primary lesion. Since then many workers have verified his finding but not his conclusion, e g , McDonald (1903), Brinckerhoff and Moore (1909) and Solis and Wade (1925). Such a high percentage of nasal infections as Sticker found is usually seen in advanced cases only, and it seems likely that Sticker's cases were of this kind. *M lepræ*, in the experience of most workers, are found more commonly in the skin than they are in the nose. The one exception to this in recent literature is the report by Wade and Solis (1927) of nasal infection persisting in 55 out of 570 cases otherwise negative after treatment.

THE IMPORTANCE OF BACTERIOLOGICAL EXAMINATION

(a) *In diagnosis* If early cases of leprosy are to be diagnosed and not missed, diagnosis must often be made when the organism cannot be found clinically. In this respect the problem of the diagnosis of leprosy is similar to that of tuberculosis. Though most cases can be diagnosed on clinical grounds alone, there are a certain

number of cases in which bacteriological examination is the only means of clinching the diagnosis

(b) *In the evaluation of progress under treatment* Diminution in the number of *M. lepræ* and the possible final disappearance of the organisms from the tissues examined are the most important criteria of progress. On such findings as the latter will often depend the isolation or otherwise of the patient.

(c) *In the comparison of results obtained by different leprosy workers*—This is only possible if accurate bacteriological examinations are made before and after treatment. Some leprosy workers publish results with no bacteriological reports at all, some publish results with bacteriological reports based on inefficient methods and some publish results with accurate bacteriological reports. The result is that on paper the most accurate and scientific workers often show the worst results.

From all these three view-points it is desirable that where possible accurate bacteriological examinations be made in every case before and after treatment, that the most efficient methods be established and put in general use, and that no results recorded without such accurate bacteriological reports be accepted as scientific evidence regarding leprosy and its treatment.

METHODS NOW IN COMMON USE

In the space available only essential details can be given

1 *Nasal examination*

(a) *Smear of nasal discharge*—A speculum is inserted in the nose and the cavity examined for any suspicious lesion. With a small piece of cotton-wool held in a forceps, the suspicious area is swabbed forcibly, and if no suspicious area is seen, the mucous membrane over the septum, the anterior end of the inferior turbinate bone and the floor of the nose is swabbed forcibly. The material obtained is smeared on a slide.

(b) *Scraping of nasal mucous membrane*—The proceeding is similar but instead of swab, a sharp pointed bistouri is used and with as little bleeding as possible a fragment of mucous membrane is scraped from any suspicious area, or, if there are no visible lesions, from the areas mentioned above. If necessary, fairly extensive areas of the nasal mucous membrane can be scraped with the edge of a blunt instrument.

2 *Skin examination*

(a) *The slit method*—In any suspicious area of skin, a slit is made through the epidermis well into the corium by means of a sharp scalpel. Bleeding is prevented by pressure with fingers on either side of the slit, any blood which has already oozed out is wiped away, and with the point of the scalpel the deeper layers of the slit are scraped until a considerable amount of cellular material from the corium is obtained on the point of the scalpel. This is smeared on the slide.

(b) *The clip method* —In any suspicious area a fold of the skin is elevated by means of picking it up with fine toothed forceps or else by transfixing it with a fine needle, and with a pair of sharp scissors curved on the flat a fragment of the skin thick enough to include the corium is snipped off. The excised skin is held with forceps on a slide and the cellular parts (i.e., the corium) are scraped off from the epidermis and smeared on a slide. The epidermis is rejected as useless for examination.

N B —From the point of view of bacteriological examination a suspicious area of skin is one that shows thickening or erythema or both. Depigmented anesthetic areas of skin rarely show *M lepræ*.

3 Ear lobe examination

This is a special form of skin examination. The ear lobe is very commonly affected in leprosy and it may be examined as a suspicious skin lesion. Experience has shown the value of ear lobe examination as a routine procedure even when there is no apparent thickening, so for the purpose of this paper, ear lobe examination is treated as a special method. Either the slit method or the clip method as described above may be used. Owing to its structure, the ear lobe is peculiarly suitable for the clip method. A snipping of the skin including the corium can be made almost instantaneously with practically no pain to the patient.

4 Other methods

When the above methods fail two other methods can be tried. They are gland puncture [Henderson (1927)], and excision of a piece of skin for sectioning. These methods are only needed in exceptional cases. They are not suitable for adoption as a routine method of examination and they will not be further discussed in this paper.

INVESTIGATION OF THE RELATIVE EFFICIENCY OF THESE METHODS

In severe cases of cutaneous leprosy the nasal mucous membrane is usually involved and in such cases *M lepræ* can be demonstrated with ease by any of the methods described. The only way to test these methods critically is to take a large series of cases of all kinds, some slight, some showing mostly neural lesions, some patients merely suspected of leprosy and some more severe cases.

Accordingly 160 such patients were chosen, all such patients as might come any day to any doctor in India for diagnosis and each one had a slide prepared by each of the following methods —

- 1 Nasal smear
- 2 Nasal scraping
- 3 Skin slit from suspicious skin lesions other than the ear
- 4 Skin clip from the same lesion
- 5 Skin slit from the ear
- 6 Skin clip from the ear

Thus 960 slides were prepared and carefully examined and an analysis of the results was made which should be of some value

RESULTS OF INVESTIGATION

- 1 Out of 160 nasal smears 45 showed *M lepra*
- 2 Out of 160 nasal scrapings 65 showed *M lepra*
- 3 Out of 160 skin slits 94 showed *M lepra* in lesions other than the ear
- 4 Out of 160 skin clips 104 showed *M lepra* in the same lesion
- 5 Out of 160 skin slits 110 showed *M lepra* in the ear lobe
- 6 Out of 160 skin clips 128 showed *M lepra* in the ear lobe

The following is a statistical analysis of the results of these 960 slides, arranged so as to demonstrate the comparative efficiency of the various methods

Nasal examination

Of 160 smears 45 showed *M lepra*, i.e., 28 per cent

Of 160 scrapings 65 showed *M lepra*, i.e., 40 per cent

No patient showed a positive smear and a negative scraping

20 patients showed negative smear and positive scraping

This shows that the nasal scraping is a more efficient method than the smear

Nasal examination and skin examination compared

Of 640 slides taken from the skin of 160 patients 436 showed *M lepra*, i.e., 68 per cent

Of 320 slides taken from the noses of the same patients 110 showed *M lepra*, i.e., 34 per cent

No patients showed *M lepra* in a nose and not in the skin

63 patients showed *M lepra* in the skin and not in the nose

Therefore skin examination is far more efficient than nasal examination

Slit and clip methods compared

Of 320 slides taken by the slit method 204 showed *M lepra*, i.e., 64 per cent

Of 320 slides taken at the same site by clip method 232 showed *M lepra*, i.e., 73 per cent

No area of skin showed *M lepra* by the slit method and not by the clip method

28 areas of skin showed *M lepra* by the clip method and not by the slit method

Therefore the clip method is a more effective method than the slit method

Ear lobe examination compared with skin examination in other sites

Of 320 slides taken from the ear lobe 238 showed *M lepra*, i.e., 75 per cent

Of 320 slides taken from the skin in other areas 198 showed *M lepra*, i.e., 62 per cent

4 patients showed *M lepræ* elsewhere and not in ear lobe

36 patients showed *M lepræ* in the ear lobe and not elsewhere

Therefore *M lepræ* are most commonly found in the ear lobe but for greatest efficiency this method should be combined with skin examination of suspicious lesions elsewhere

Summary of results

Nasal examination showed bacilli in half the number of cases in which they were shown by skin examination. Nasal scraping is more efficient than smear. Skin examination by either slit or clip method is far better than nasal examination but the greatest number of positive results is obtained by the clip method in the ear lobe. Four cases showed bacilli elsewhere and not in the ear lobe.

DISCUSSION OF RESULTS

It is not suggested that in any cases the ear lobe is the only area in which *M lepræ* occur, but these findings indicate clearly that the ear lobe is usually the area in which *M lepræ* are most easily demonstrated clinically. A considerable number of cases giving positive findings in the ear lobe showed no visible thickening or erythema of the ear lobe. Some of these cases are illustrated in the accompanying photographs. What apparently happens is this. Before the development of visible skin lesions, a few bacilli lodge in the corium of the skin in various parts of the body and can be demonstrated clinically if a good exposure of corium is obtained at examination. The clip method gives a better exposure of corium than the slit method and in the soft pendulous ear lobe a good exposure of corium is most easily obtained. In other areas of skin commonly affected in leprosy, e.g., the brows and the chin, the skin is much thicker and denser and a good exposure of corium is much more difficult to obtain.

How are these findings concerning the relative frequency of positive findings in the nose and skin reconciled with the findings of Wade and Solis that after treatment 9 per cent of 570 otherwise negative cases showed *M lepræ* in the nose. Many of these 160 patients had been receiving treatment and in these cases similar findings might have been expected. It was not so. Not one case showed *M lepræ* in the nose and not in the skin also and 63 showed *M lepræ* in the skin and not in the nose. This is in accordance with the writers' experience for several years. We have never seen a case before or after treatment which showed bacilli in the nose only. The explanation of the difference of these findings probably lies in the difference of technique of examination used. In the Wade and Solis series of cases, the scraping method in the nose and the slit method in the skin were used. In the present series, if these methods are used 6, or 4 per cent, of patients show *M lepræ* in the nose and not in the skin, but if the clip method is used in the ear lobe none show *M lepræ* in the nose and not in the skin also.

Notes on interesting cases showing M lepræ

(See Plates XLVII and XLVIII)

- Case No 1* B J, age 20 Father is an infective leper and son had very slight depigmentation of face and back No anæsthesia, no apparent thickening of skin, but some anhydrosis of limbs Skin of ear lobe showed a few *M lepræ* by clip method only
- Case No 2* N M, age 25 Father and two sisters lepers There was very slight anæsthesia of his foot The skin of his face was marked by small pox scars but there was no definite thickening The scraping of mucous membrane and the skin of the ear lobe showed *M lepræ* in large numbers The patient is an example of a highly dangerous and infectious type of leprosy There was no obvious disease yet he was discharging millions of bacilli daily from the nose
- Case No 3* F, age 25 A State prisoner who developed a slightly depigmented patch on the arm and was sent for examination by the prison doctor No apparent thickening of the skin anywhere but a clip from the ear lobe showed *M lepræ*
- Case No 4* P B, age 30 No family history of leprosy, but there were some lepers in his village Had some formication in limbs followed by appearance of some depigmented patches No apparent skin thickening anywhere but skin of ear lobe showed *M lepræ*
- Case No 5* K L, age 30 No family history of leprosy, but some lepers were in his village First noticed tingling followed by anæsthesia of index and middle finger of right hand No other signs of leprosy are found No apparent thickening of skin anywhere Skin of ear lobe showed *M lepræ*
- Case No 6* N R, age 12 No family history of leprosy Six months ago noticed a depigmented anæsthetic patch on the leg No other signs of leprosy, no apparent skin thickening Skin of ear lobe showed *M lepræ*

Negative cases

Of 160 patients, 132 showed *M lepræ* on examination by the methods described Of the 28 negative cases, 20 were diagnosed as leprosy on clinical grounds alone Of these 20 one showed *M lepræ* on excising a piece of skin for section and one showed *M lepræ* in an abscess of the ulnar nerve at operation In the remaining 8 cases, mostly 'contacts,' a diagnosis of leprosy was not made

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Case No 1



Case No. 2



Case No 2(a)



Case No. 3.



Case No 4



Case No 5



Case No 6

EDIBLE AND PARALYSIFIC BUGS, ONE OF WHICH
A NEW SPECIES *CYCLOPelta subhimalayensis*
N. SP (*HEMIPTERON*, *HETEROPTERON*,
PENTATOMIDA, *DINADORINA*)

BY

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[Received for publication, August 10, 1931]

EDIBLE bugs of the species *Aspongopus nepalensis* Westw 1837, have been referred to in Distant (1902) who citing O Gorman, Major, I M S , says that certain natives of Assam are accustomed to eat them pounded up with their rice, a statement passed on by Maxwell-Lefroy (1909) who remarks that probably the insect gives a powerful aromatic flavour to the diet

It was a related bug that was subsequently (February 10th. 1930) sent to the writer by Dr O'Connor of Upper Assam with the following note "I send herewith a beetle which was found in one of the Assam Frontier Tracts It lives under large stones in the Lohit River and appears to be of an edible type The Mishmis eat it but before doing so they remove two little red bags which they say contain poison these bags appear to lie between the thorax and abdomen On mentioning this matter to some gentleman whose name I have unfortunately forgotten he informed me that he knew of it and that if perchance the natives forget to extract the poison glands they get paralysis of the neck from which they inevitably die "

The species sent by Dr O'Connor was kindly identified for the writer by Mr W E Chna, British Museum, as *Aspongopus chancensis* Dall, 1851

In addition to the above cited reports regarding the edibility of these bugs (or so-called ' beetles,' or more specifically ' cinnamon beetles ') [other notes have amply confirmed the matter to the writer Dr Hutton, I C S , Commissioner, the Naga Hill Tracts , Mr Mills, I P , Acting Commissioner, and citing Mr Needham

of Upper Assam, Mr Furze, I R, Political Officer of the Sadiya Frontier Tract, and Mr W C M Dundas, C I E, late Inspector-General of Police, Assam, have all sent letters to the same effect

Mr Furze, moreover, sent the writer a large number of specimens of the bug and they have proved to be of three species, not only the *Aspongopus chinensis* mentioned above and *A. nepalensis* as noted in Distant, but also a new species *Cyclopelta subhimalayensis* which will be described below

The tribes reported to have the custom are the Miris, Mishmis, Abors, and some Nagas. In the case of the Mishmis, Mr Furze explains the addiction on the ground of their living a meagre existence in very precipitous country

Reports of these natives abstracting a poison gland and of its action on those who have eaten it have been remarked upon by the above gentlemen as follows. Mr Mills, 'there are no tribes in these (Naga) hills who extract the poison glands from bugs, but Mr Needham says that on the north bank of the Brahmaputra the Abors and Miris extract a small gland from the neck of the beetle in the belief that the gland if eaten affects the brain

Mr Furze recollects hearing a well-authenticated report of a beetle that was believed to cause paralysis. 'The beetle has internally near the head a small red spot which is said to be very poisonous and to cause madness,' but in his own experience he had never seen a case of paralysis, though symptoms of poisoning ensued if eaten by individuals not accustomed to the diet. Mr Furze cites the Abors as saying that occasionally "an exceptional beetle 'with a spirit' is eaten which causes paralysis.' Mr Dundas also confirms that if people unaccustomed to eating the beetle do so they become violently sick

The beliefs and superstitions here recounted have been the subject of some investigation at the School of Tropical Medicine, Calcutta, for which purpose the writer has utilized the ample material so kindly sent by Mr Furze

In the first place the so-called poison-gland *alias* the heart was found to be the stink-gland. It is a scarlet-coloured bi-lobed median sac lying between the abdomen and meta-thorax. It can easily be demonstrated on the ventral aspect by merely fracturing the thorax-abdominal articulation on bending the abdomen back on the thorax. The contents of the sac are highly volatile oil

An attempt was then made to find out whether the ingestion of the entire bug without abstracting 'the heart' had such a paralytic action as it was purported to have. For this purpose they were fed to monkeys, but these did not become paralytic

The glands were then dissected out and a saline 'emulsion'—the volatile oil floating on saline—injected intravenously was tested on cats. These tests were kindly carried out for the writer by the Pharmacological Department of this Institute, under the charge of Colonel Chopia, I M S. The reaction on the respiration and circulation was quite negative

With regard to the report regarding the action of the contents of the heart or the skin not only was an alcoholic extract of the glands painted on the skin without effect but the whole gland dissected out was applied to the skin without any more result

One is compelled then to conclude that in spite of the popular belief to the contrary the stink-gland or the heart has none of the physical attributes ascribed to it and the origin of these beliefs can only be wondered at. It remains to be seen whether the statement of the Abors that if one unluckily picks on a bug possessed of a spirit one becomes afflicted of a puls is any the more correct.

The following is the description of the new species —

Cyclopelta subhimalayensis n. sp. (Hemipteron Heteropteron Pentatomida
Dinadorina) —(see Plate XLIX)*

FORM ovate, dimensions overall —length ♀ 25 mm ♂ 23 mm breadth between pronotal angles ♀ 14 mm ♂ 13 mm

HEAD small with apex truncate lateral lobes a little longer than central and meeting beyond it. Proboscis nearly reaches the middle coxa. Antenna with fine pale hairs and fewer dark spines, 4-jointed the segments in length being relatively about 1 4, 3 3 the basal joint passing the apex of head.

THORAX Pronotum dark brown obscurely wrinkled lateral angle sharp but not produced, mesosternum sulcate scutellum dark-brown about 2/3rds of length of abdomen, lateral margins concave apex broad and blunt wings, complete, hemelytra brownish-yellow, membrane large, paler primary and subtended veins distinct and converging at the apex, posterior wings paler yellow.

ABDOMEN unarmed at base, connexivum dark-brown monate, body above, reddish-orange, beneath, brown legs bright brown.

The type ♀ ♂ will be lodged in the Indian Museum and paratypes in the School of Tropical Medicine and Central Research Institute, Kasauli.

For the convenience of diagnosis from the other species reported edible, a short account of their characteristics (Distant) is given here —

Aspongopus nepalensis

Dark-brown ochraceous or pale castaneous—extreme lateral margins of pronotum black antennæ black, apical joint luteous, narrowly black at base, body underneath and legs more or less cupreous. Antenna 5-jointed, 2nd and 3rd joints of antennæ sub-equal in length, abdomen above red.

Length 20 to 25 mm

Breadth between pronotal angles 11 to 13 mm

*The writer is much obliged to Mr V. Nag, one of the School artists, for the trouble he has taken with this drawing.

A chinensis

Bronzy purplish-black, connexivum black, with transverse narrow dull reddish spots at the middle of the segments, antennæ 5-jointed pilose, black, apical joint ochraceous, its base black, second joint much longer than the 3rd, 4th joint distinctly furrowed

Body above very finely and obscurely punctate, posterior tibiæ slightly dilated near base, body beneath more distinctly punctate than above

ACKNOWLEDGMENT

I would like to thank Mr Ribiero of the Indian Museum for assisting me in going over the Museum collections

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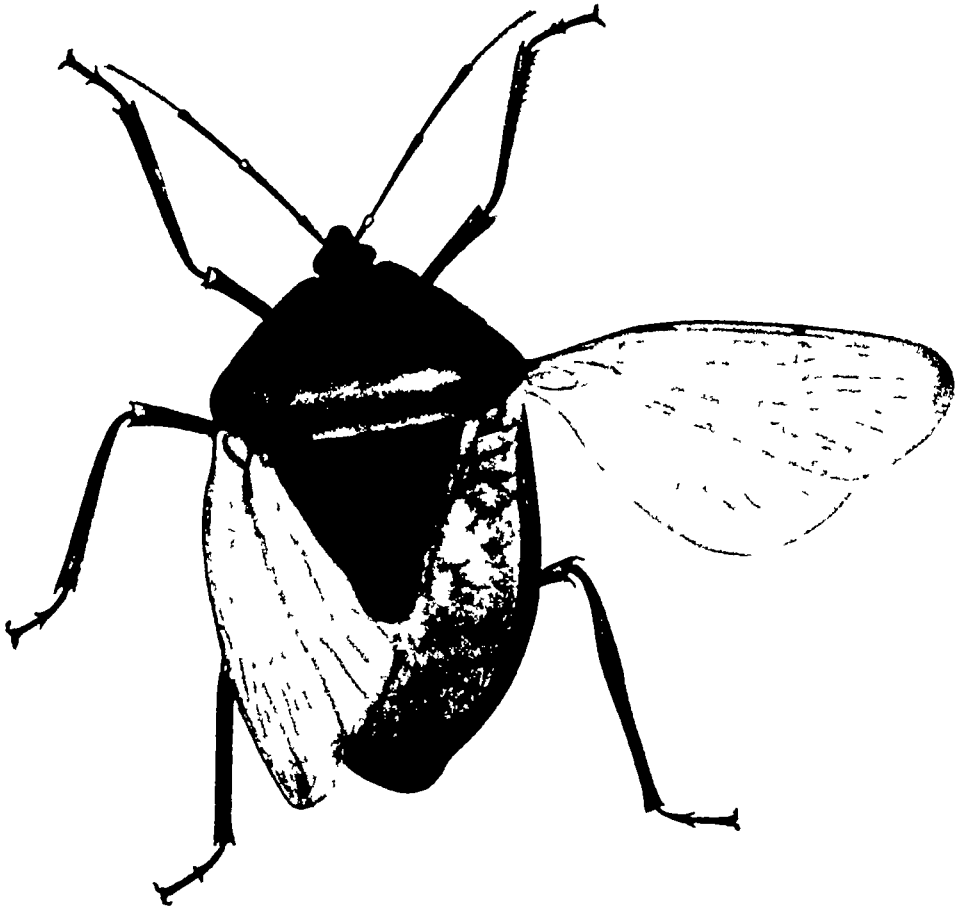
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PLATE XLIX.



Cyclopelta subhimalayensis n. sp.

ROLE OF ADRENALINE IN MAINTAINING THE NORMAL TONUS OF ORGANS OF THE BODY

BY

N K BASU, M B,

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[Received for publication August 22, 1931]

THERE is a great deal of controversy about the point whether the supra-renals are glands of emergency only or whether the secretion is continuous Sharpey-Schaffer has thus summed up—'on the whole the evidence is in favour of the view that there is not only a normal and fairly constant passage of adrenaline into the blood but it may be temporarily increased under conditions of nervous excitation'

Now if this secretion be continuous, three main questions remain to be solved—

- (i) How is adrenaline carried in the blood?
- (ii) What is the utility of this continuous secretion?
- (iii) How does adrenaline act?

A In testing for adrenaline in the blood O Connor (1912) states that plasma and not serum must be employed But according to the view of Sajous (1918), the whole of the adrenaline is carried by the corpuscles and not by any other portion of the blood

No doubt, nearly the whole of adrenaline is in the corpuscles but the reasons for this are —

(i) Adrenaline remains in combination with the protein portion of the blood and the protein portion of the corpuscles is the greatest*,

(ii) Adrenaline being a base it remains in combination with some acid and the iso-electric point of the corpuscles is the greatest †

* Protein content of

(a) corpuscles	31 per cent
(b) plasma	9 „ „
(c) serum	8 „ „

† Iso electric points of

(a) oxyhemoglobin	6.8
(b) plasma	5.9
(c) serum globulin	5.4
„ albumin	4.7

Experimental evidences in support of the assumption that the whole of the adrenaline remains in combination with the protein portion of the blood and that nearly the whole of adrenaline is in the corpuscle-protein are —

The most delicate qualitative test for adrenaline appears to be its effect on intestinal muscles. For this rat's intestine seems to be a very suitable one, as it shows definite relaxation with a dilution of 1 in 1 billion. But for several advantages which frog's intestine possesses over rat's intestine, it was selected for use throughout these experiments.

(i) The effect of adrenaline in very small doses is to produce a contraction and thus the effect reproduced on the graph is much better read,

(ii) Solution of adrenaline even in a dilution of 1 in 500 millions shows contraction,

(iii) Being a cold-blooded animal perfusion can be very easily carried on with normal saline only and without any further complication.

For carrying out these experiments a miniature Dale's perfusion apparatus was specially made, the capacity of the inner bath being 6 c.c. only. Samples of blood were collected by puncture of the heart of the rabbits.

The effects of plasma, serum and corpuscles were tested on intestine separately, and the efficacy of these three were found to be in the following proportion —

Corpuscles Plasma Serum 1 1/2,000 1/200,000 *

From each of these protein was precipitated with trichloroacetic acid and it was found that protein-free filtrate in each case had no action. But the precipitated protein diluted with normal saline produced contraction. Here also, protein obtained from the corpuscles showed the greatest effect.

To test the presence of adrenaline in this precipitated protein for the contraction of intestine, two different methods were adopted.

In one series, effects of ordinary protein (lecithin being used) and non salts were tested on frog's intestine and the change of effect on addition of adrenaline to those solutions was also noted.

In the second series, the effect of adrenaline was first tested and the change on addition of protein and non salt solutions to adrenaline was also noted. In both these series, adrenaline was added in a dilution of 1 in 100 millions.

From both these series of experiments, it was found that combination of adrenaline with protein and non salt solutions produced the maximum effect. Even in those cases, where the dilution of protein and non salt solution produced no effect on the intestine, addition of adrenaline at once produced the desired effect.

* (i) Serum 1 in 500 contracts the frog's intestine

(ii) Plasma 1 in 50,000 contracts

(iii) Corpuscles 1 in 100 millions contracts

Solutions of oxyhæmoglobin and methæmoglobin were also tested separately, and it was found that oxyhæmoglobin solution produced some effect but not the solution of methæmoglobin. Moreover, it was found that addition of oxygen to the solutions of protein and iron, or to the solution of protein, iron and adrenaline, did not improve the condition of the perfused intestine.

B. About the utility of this continuous secretion of adrenaline. Sajous advocated (1918) and Menton corroborated (1923) the belief that adrenaline plays an important part in assisting the combination of oxygen with hæmoglobin, and it is this combined oxygen which is responsible for all the actions of adrenaline.

From the present series of experiments, while it seems to be true that adrenaline plays an important part in assisting the combination of oxygen with hæmoglobin, no support could be found for the belief that this combined oxygen is solely responsible for all the actions of adrenaline.

On the other hand it might be suggested that the presence of adrenaline is of absolute necessity for hæmoglobin to carry out its normal function.

The continuous secretion of adrenaline can be said in general to serve to keep up the normal tone of different systems. This normal tone is kept up in two ways—

(i) directly, (ii) indirectly

and these two processes work simultaneously

What is meant by the indirect way of keeping up the normal tone is best explained by an example. The normal effect of adrenaline on the intestine and lungs is to inhibit their actions, and here adrenaline indirectly helps to keep up the normal tone of the intestine and lungs by antagonizing the effect of choline which is also normally present in these tissues and which is always stimulating these tissues.

High dilution of adrenaline circulating in the blood would have no obvious effect on the blood-pressure but here also the normal tone of vessels is kept up mainly by directly stimulating the muscle fibres and partly by indirectly antagonizing the effect of choline.*

In order to test this indirect way of keeping up the normal tone of the systems by adrenaline, supra-renal glands of a number of guinea-pigs were put out of function by injection of minute quantity of carbolic acid into the gland after opening the abdomen under general anæsthesia. Most of the animals did not survive the operation and died within 24 hours. But a few survived and on the third day these animals were killed and loops of intestine and uterus were taken out for recording the movements. Neither intestine nor uterus showed normal movements but on

* Amount of choline in various tissues (after Kinoshita)

1 Small intestine	0.022 to 0.030	4 Pancreas	0.015 to 0.03
2 Kidney	0.020 to 0.029	5 Spleen	0.012 to 0.03
3 Muscle	0.016 to 0.032	6 Lung	0.016 to 0.011

the contrary the movements were extremely irregular and the muscles were in a state of permanent tonic contraction

And from these experiments it can be safely concluded that adrenaline secreted in the body helps to keep up the normal tone of the systems by neutralizing the effect of choline which is also present in the tissues normally

So the relation of adrenaline to choline in our system can be summed up thus—where choline acts as a preponderant hormone the antagonistic effect of adrenaline is a depressant, and where choline influence is small or nil adrenaline is not a depressant but a stimulant *

U Regarding the mode of action of this circulating adrenaline It has been suggested from the peculiar relation of adrenaline to the sympathetic system that adrenaline acts by stimulating this system But that such is not the case is shown by experimental evidences, for tissues supplied by severed nerve, especially after its fibres are degenerated, are more easily excited by adrenaline Nor, regarding the suggestion that minute quantity of adrenaline circulating in the blood may serve to maintain or increase the irritability of sympathetic nerve-endings, can any experimental support be adduced, for animals deprived of supra-renals react as readily as normal animals to sympathetic excitant (Hoskins, 1914)

While it is true that adrenaline stimulates the sympathetic nerve-endings or the flow of adrenaline secretion is regulated by various factors, this is not the sole mode of action of adrenaline As has been said before, it is a general excitant and its presence is of absolute importance for the life of the individual cells of our body and it acts as a stimulus to these individual cell-lives

And how does adrenaline act as a stimulus to these individual cell-lives ?

It enters into some form of chemical composition with the physiological units on which it has action—thereby some transformation of energy is caused And this energy-transformation acts as a stimulus

Now it may be questioned, if this secretion of adrenaline be of such vital importance, how can the individual cell-life continue its function when the supra-renals are not functioning ? In answering this question we must remember that the 'adrenal system' does not comprise only a pair of glands but also there is a close relation between this adrenal system and other glands of our body. So that even when this pair of glands is out of function, the rest of the adrenal system and the related glands go on continuing this work But in such cases survival of life

* Physiological effects of choline —

- (i) It circulates in the blood in a dilution of a 1 in 100,000 to 400,000
- (ii) On the heart, it exerts a weak muscarine effect
- (iii) On the blood pressure, it produces a fall
- (iv) On the intestine it increases the vagus tone
- (v) On the uterus, it increases the tone
- (vi) On the eye, it acts like pilocarpine

is only temporary and varies in its duration in different species of animals according to the development of this adrenal system and to the establishment of inter-relation between the adienal system and other glands of the body

So in old age when the secretion of adrenalne is decreasing gradually, every system of our body continues to lose its tone and the secretion of other glands also continues to diminish simultaneously Even in general diseases when the secretion of the adrenalne glands is rapidly exhausted the longevity of the individual cell-life is correspondingly diminished

STUDIES ON INDIAN SIMULIIDÆ

Part I.

SIMULIUM HIMALAYENSE SP N , *SIMULIUM GURNEYÆ*
SENIOR-WHITE , AND *SIMULIUM NILGIRICUM* SP N

BY

I M PURI, M SC (Punjab), PH D (Cantab), F E S ,
*In charge, Inquiry on the Indian Simuliidæ, Culicoides and other
Blood-Sucking Midges*
(From the Central Research Institute, Kasauli)

[Received for publication, September 9, 1931]

SINCE Brunetti (1911) described a number of Indian Simuliidæ very little has been done on these insects in India, the only other works dealing with them being descriptions of females of three new species by Senior-White (1922) and two by Edwards (1927) The former described these species from Coonoor (South India) and the latter from specimens sent to him from North-East Kashmere Nothing has, however, been published so far on the early stages in the life history of the Indian Simuliidæ

During recent years, while working on the larvæ of the Indian anopheline mosquitoes, I had occasion to collect and rear from pupæ a large number of simuliids from various parts of India and a study of this material has shown that the descriptions of many species, already described, are inadequate and may equally well apply to more than one species of *Simulium* occurring in this country Consequently it has been thought advisable to give, besides descriptions of new species, revised descriptions* of species already recorded from India, giving also such

* It was to give adequate descriptions of the various species and to deal with the large collection which had accumulated that an inquiry on the Indian Simuliidæ was started under the Indian Research Fund Association

characters as may later on be useful in making a comparative study of the group. Moreover, as most of the specimens in my collection have been bred out from isolated pupæ, it has been possible for me to correlate the males of the different species to their respective females and vice versa.

Edwards (1931) while dealing with the Simuliidæ of Patagonia and South Chile has given definitions of the various subgenera into which the genus *Simulium* can be divided. Some of these subgenera, according to him, are the only ones that can safely be retained (only as subgenera) out of the large number of genera into which Enderlein has broken up the family. A casual study of the collection at my disposal has shown that most of the Indian Simuliidæ fall into the subgenus *Simulium*, the rest coming under *Eusimulium*, but at this stage it is too early to say as to how far the Indian species conform to the definitions of the subgenera as given by Edwards. It is intended to follow the classification as given by him till such time as a detailed study of the Indian Simuliidæ shows any discrepancy in the definitions.

Edwards has at the same time described the various external characters which serve as the main diagnostic features for the identification of the different species and these need not be described here. The only structures, which owing to a great deal of confusion in the terminology of the various parts composing them, need a special reference here are the terminalia of the male and female. Pending a more detailed description of these parts I give below a brief outline of these structures sufficient for the present purpose*.

The terminalia of male simuliidæ (Plate L, fig. 5) consist of the ninth segment carrying a pair of appendages, modified tenth and eleventh segmental structures—the *proctiger* (Freebone, 1924)—carrying cerci, and thirdly a median genital mass. The ninth tergite is large forming an expanded plate and ventrally there is a narrow bat-shaped sternite (s9). Each appendage consists of a short basal portion, the *coxite* (side-piece) (C) and a usually long massive terminal portion, the *style* (clasper) (s). The proctiger has dorsally an oval plate, the tergite of segment 10 (t10) and towards its apex, on either side of the membranous anal opening a small hair-bearing cercus (Cc). Whilst anterior to the cercus and smaller is on either side a hair-bearing protuberance—*paraprocts* (Pp). The genital mass, which is very large and fleshy, consists of an anterior portion, termed by Edwards the *ventral plate*, which is frequently shaped like a median flattened hook and lies between the bases of the coxites—the *inter-coxal piece* (H), and a more posterior mass springing from the ventral aspect of the proctiger—the *mesosome*, and carrying the genital opening (O) at its apex, with on each side of this a conspicuous line of strongly curved spines. Ventrally on the mesosome is a chitinized

* I am grateful to Col. Sir S. R. Christophers for his help and suggestions in regard to the homologies and nomenclature given.

plate which is continued proximally in a curve on to the inter-coxal piece. In the various species the parts which mainly show specific differences are the form of the inter-coxal piece, the size, form and structure of the styles and the relative size of the coxites.

In the female the terminalia (Plate L, fig 1) consist of a but little modified segment 8, bearing posteriorly a pair of appendages, the *anterior gonopophyses* (*AG*), a ninth segment with a chitinized tergite and membranous ventral area, and a modified tenth and eleventh segmental mass—the *proctiger*—carrying cerci. The eighth tergite and sternite are well developed, the former bearing fine setæ all over while the latter (*s8*) shows these usually on its lateral portions only, the median area being smooth. The pair of appendages, the anterior gonopophyses (*AG*) arise intersegmentally one on each side of the median line and are directed backwards in the form of a pair of flattened plates separated by a narrow interval. Dorsal to the appendages is the horizontal slit-like opening of the oviduct. Lying on the membranous intersegmental area arising on either side from the lateral arms of the ninth tergite (*t9*) and passing as a median chitinized rod along the oviduct is a Y-shaped chitinization, the *furca*. The ninth tergite is large, giving attachment at its narrow ventral terminations to the arms of the furca. The proctiger, as in the male, has an oval plate dorsally, the tenth tergite (*t10*) and on either side of the membranous anal opening a flattened scoop-shaped hair bearing cercus (*Cc*). Arising on each side of the anal opening and lying ventral to the cerci are a pair of rounded strongly chitinized protuberances, the paraprocts (*Pp*). These seem to be superficially divided into two unequal parts, a smaller interno-anterior and a larger external and posterior. In the various species the terminalia show differences mainly in the form of the eighth sternum and of the anterior gonopophyses, the size of the furca, and the form and relative size of the paraprocts and cerci.

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***Simulium (Simulium) himalayense* SP. N**

FEMALE

Head black, with fairly dense short black hairs on the occiput, but scanty on the face and only a few along the lateral borders on the frons. Frons nearly black, distinctly shining, nearly rectangular, only a trifle narrowed just above the region of the antennæ, about one and a half times as long as its greatest breadth near the top. Face conspicuously dusted with silver grey. Antennæ with the scape and the two basal flagellar segments orange (varying in colour in the paratype specimens from yellow to orange), the rest becoming black gradually and having very fine pale grey pubescence. Palpi black.

Thorax mostly black, somewhat shining, the anterior one-third with slight grey dusting, which is deeper in the antero-lateral angles of the mesonotum and

which when viewed from above appears as two rounded spots, one in each angle Mesonotum is densely covered with coarse golden pubescence Scutellum also with coarse golden pubescence and black marginal hairs Pleurae and sterna slate-grey, membranous area of pleurae bare

Abdomen The first segment yellowish, with the fringe of long hairs golden The second tergite brownish yellow with silvery grey dusting Segment 3 dark brown, the rest nearly black (The colour of the abdomen varies in the different paratype specimens from black to dark-brown) The segments posterior to segment 4 with short, scattered dark hairs, a few being present also on segment 4 dorsally The tergites of segments 6 to 8 shining Terminalia (Plate L, fig 1) Setae on the ventral surface of segment 7 are evenly spread out and are more or less adequal in size Sternite of segment 8 is somewhat rectangular in shape with a more or less straight posterior border The anterior gonopophyses (AG) bear a large number of fairly stout setae scattered all over, have a somewhat thickened inner border and are shaped as in Plate L, fig 1 Paraprocts (Pp) are thinly chitinized and are of moderate size and the cerci (Cc) also are fairly small

Legs Fore coxae and trochanters pale-yellow, femora deep-yellow with the tip dark, tibiae yellowish, the distal one-fourth black, the outer side with a large silvery patch, tarsi (Plate L, fig 2) black, moderately expanded, first segment about five times as long as it is broad at the distal end (Plate L, fig 2), segments 1 and 2 together a little shorter than the tibiae Segments 1 and 3 with a pair of long black hairs subapically on their posterior border Middle and hind coxae blackish, trochanters golden yellow, femora golden yellow gradually darkened near the tip, tibiae mostly golden yellow, dark near the distal end, with a large silvery patch on the basal two-thirds of the posterior surface, tarsi about equal in length to the tibiae Most of the first, basal half of the second and the base of the third tarsal segment of the middle leg and basal two-thirds of the first and base of the second tarsal segment of the hind leg yellowish, the rest of the tarsi black All femora tibiae and yellowish parts of the tarsi with golden pubescence Pedisulcus* (P) well marked, calcipala* (Cp) of moderate size (Plate L, fig 3) All claws with a sub-basal tooth (Plate L, fig 4).

Wings normal, hyaline, radius bare up to the fork Radial sector is simple and is a concave vein bearing setae on its under surface but none above Halteres pale yellow

* *Pedisulcus* is the name given by Enderhen to the dorsal incision near the base of the second segment of the hind tarsi and *Calcipala* to the flattened projection at the tip of the first segment (basitarsus) of the hind tarsus, extending beyond the base of the second segment on the inner, and not the outer side, as erroneously described by Edwards (1931)

Wing length 3 mm, varying in many paratype specimens from 2.5 to 3.1 mm

The ventral surface of the buccal cavity with a small cluster of minute nodules at its posterior end

Spermatheca single, globular in form and uniformly dark brown

MALE

Head black with a fringe of short dark hairs on the occiput, face whitish grey with scanty black hairs. *Antennæ* black with very fine pale pubescence. *Palpi* black.

Thorax velvet black, covered uniformly and densely with coarse, short golden hairs, and also long scattered black hairs on the prescutellar region. Anteriorly are a pair of silvery spots broadly separated in the middle. These spots are seen complete and not in halves, like those found in a similar position in *S. ornatum* Meigen. In certain lights the mesonotum shows a broad silvery border laterally and posteriorly. This silvery band is continuous with the anterior spots. *Scutellum* black, covered with coarse golden hairs and a fringe of black hairs. The membranous area of *pleuræ* bare.

Abdomen velvet black with scattered short golden hairs on it. Silvery lateral spots on segments 2 and 5-7, those on segment 2 brightest of all and connected with each other by a silvery spot along the anterior border of the segment dorsally. The long hairs, on the basal scale black basally, gradually becoming golden brown towards their tips. *Genital armature*. The genital armature (Plate L, fig. 5) to some extent resembles that of *S. ornatum*. The coxites (*C*) are short, nearly as long as they are broad, the styles (*s*) are rather long, nearly three times as long as their width at the base, of more or less the same width throughout their length (in some specimens their distal end is, however, comparatively narrow). Each of them bears a single short spine subterminally on the inner edge. The inter-coxal piece (*H*) has a fairly broad base, from which a thumb-like process projects downwards and curves forwards with somewhat toothed lateral edges in the proximal half. The distal half of the intercoxal piece is much thinner and bears fine setæ on its outer surface.

Legs. Fore coxæ greyish yellow, trochanters and femora golden brown, dark at their distal ends, both covered with a short coarse golden pubescence. The colour of the trochanters and femora varies in the different paratype specimens from golden brown basally to dark brown. Tibiæ and tarsi nearly black, the former with a large silvery spot on the outer surface, tarsi moderately flattened. The middle and hind legs nearly all black. Middle tibiæ with silvery white dusting on the outer surface basally. The basal half of the mid and hind basitarsi and the base of the second segment of the hind tarsi golden brown. Basitarsus of the hind

leg only moderately enlarged (Plate L, fig 6) It is a little shorter than the tibia and its greatest width is about 0.7 of that of the latter

Wings as in the female

PUPA (Text-figure 1)

Size about 3.3×1.1 mm

The integument of the head and the thorax is light brown, with minute disc-like tubercles scattered all over. The head bears the usual three pairs of short sensory hairs, the *trichomes*, and there are four pairs dorsally and six pairs laterally on the thorax. The submedian group of *trichomes* seems to have only two hairs on each side, unlike most of the European species in which three pairs are present (see Puri, 1925, p. 332). The dorsal hairs are moderately long and are simple.

Dorsally on the abdomen there is a row of six to seven sensory hairs (four of which are often comparatively stouter than the rest) on each side of segment 2, and on segments 3 and 4 in place of the four hairs, nearest the mid-dorsal line, there are on each side four strong cuticular hooks pointing forwards. On segments 7 and 8 there is a row of backwardly directed cuticular spines along the anterior border, a few minute spines being present on segment 6 also. Segment 9 has a pair of poorly developed sub-terminal spines and a row of poorly formed spines along its anterior border.

On the ventral surface segments 5-7 bear on each side a pair of strong cuticular bifid or trifid spines bent forwards. One of them is usually simple on segment 5. There is no such spine or thickened sensory hair on segment 4 ventrally.

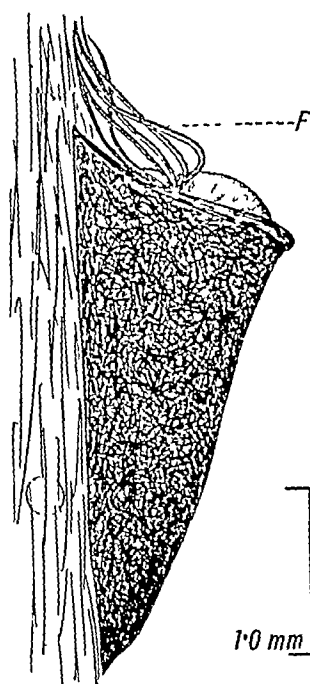
Respiratory filaments (Plate L, fig 7) are about half as long as the pupa, 6 in number, arranged in three pairs, the upper and the lower pairs with short stalks while the middle pair arises practically directly from the main stem. The filaments all spread out near their origin and the uppermost filament is more or less at right angle to the lowermost one. The surface of the filaments is covered with fine tubercles and ridges which are arranged as in Plate L, fig 8.

The *cocoon* (Text-figure 1) is about 3.5×1.2 mm, not covering the pupa completely as its length at its dorsal end is only about 2.5 mm. It is brown in colour, of the ordinary type, wall-pocket shape and loosely woven particularly in the anterior half but has no 'windows' or wide spaces in the web. Its anterior margin is thickened into a moderately strong rim. There is no floor in the anterior half of the cocoon.

This species closely resembles *S. hackeri* Edwards (1928)* from Malay and *S. gurneyæ* Senior-White from peninsular India. From the former it differs in

* I am indebted to Dr F. W. Edwards, sc. d., British Museum (Natural History), London, for sending me a paratype specimen of *S. hackeri* for examination.

having a slightly shining thorax and in its having very little dark colour on its legs *S. guineyæ* (female), on the other hand, has a comparatively much more shining mesothorax and the structure of its pupa is quite distinct. The pupa and the cocoon of *himalayense* closely resemble those of the European species *S. erythrocephalum* De Geer (Puri, 1925, p. 350).



Text figure 1

Simulium himalayense sp. n. Lateral view of pupa in cocoon
F — Respiratory filaments

DISTRIBUTION

I have bred out this species in large numbers from pupæ collected from the following places. Small streams around and in Simla (Stream below Annandale, height about 6,000 ft, 3 vii 26, Samri Nala, about 5,500 ft above sea level, 21 vii 26, Stream North of Chhota Simla, about 6,000 ft, 6 ix 30, and Chadwick Falls about 5,500 ft, 7 ix 30), in the hills (heights above sea level ranging between 4,000–5,500 ft) around Kasauli, September 1926 and October 1929, from streams near Fagu (8,000 ft), Theog (7,000 ft) and Matiana (8,000 ft) all on the Hindustan-Tibet Road (13 ix 30), and from streams in Kurseong (nearly 5,000 ft) and in Darjeeling (7,000 ft) both in N. E. Bengal (August 1928).

There is a specimen (♀) in the Indian Museum, Calcutta, from Paresnath, West Bengal (4,000–4,400 ft) (N Annandale, 12 iv 09) and one (also a ♀) in the Pusa Museum collection, marked Simla, X II

Types for the present in my own collection

***Simulium (Simulium) gurneyæ* SENIOR-WHITE (1922)**

From the description of the female given by Senior-White this species can easily be identified from among the different species found in and around Coonoor but some difficulty is apt to arise when dealing with simuliids from other parts of India. I have bred out over 300 specimens both males and females from pupæ, collected from Coonoor and some other parts of India and as a certain amount of variation in the colour of the legs has also been observed it is advisable to give a revised description of the female

FEMALE

Head black, with short black hairs on the occiput and the face. Frons nearly black, distinctly shining, slightly narrowed above the antennæ, with a few fine somewhat golden hairs along its lateral and dorsal borders. Face dusted with silver grey. Antennæ with the scape and two basal flagellar segments varying in colour from yellow to orange or reddish brown the rest dark brown to black with very fine greyish pubescence.

Thorax nearly black, somewhat shining with silver-grey dusting in the fore corners forming an indefinite pattern. Usually there is no other greyish dusting on the dorsum of the thorax but if present it is in the form of three faint greyish stripes on the anterior two-thirds of the mesonotum. The whole of the mesonotum is covered with somewhat coarse golden pubescence. Scutellum with dense golden pubescence and long black marginal hairs. Pleuræ ash grey to dark grey, membranous area of pleuræ bare.

Abdomen The first segment brownish, with a fringe of long pale golden hairs, the second segment dark-brown to black, with a large silvery grey spot and the rest black. Dorsum of segments 6–8 shining and sparsely covered with very fine pale hairs. Base of venter yellowish to brownish. Terminæ as in *himalayense*.

Legs In most of the specimens front coxæ, trochanters and femora yellow. Fore tibiæ yellow, gradually darkened in the distal one-third, with a large silvery spot on the outer side, tarsi black, moderately expanded, the first segment five times as long as its greatest breadth near its distal end. Segments 1 and 3 with a pair of long black hairs at their distal ends externally. Middle and hind coxæ nearly black, trochanters and femora yellow, tibiæ yellow with a dark distal end, basally with a whitish sheen on the posterior surface. Basal two-thirds of the

first and the bases of the second tarsal segments yellow, the rest black. The first segment of the hind tarsus is only a trifle shorter than the hind tibiae and a little more than half its width. All femora, tibiae and yellow parts of the tarsi with golden pubescence. Pedisulcus well marked, calipala of moderate size, extending up to the pedisulcus. All claws with a sub-basal tooth (which appears to be smaller than that in *himalayense*).

A few of the specimens of this species from Kodikanal (Palni Hills, S India) show a slight variation in the colour of their legs. In these the trochanters, femora and tibiae instead of being pale or bright yellow, as is usual in this species, are slightly greyish yellow, the femora have dark distal ends and the tibiae have more dark pigment than usual. The front tibiae are dark even at their proximal end. In such specimens the thorax too seems to have more grey dusting on the anterior half of the mesonotum.

Wings normal, hyaline, radius bare up to the fork, radial sector simple, concave vein, having setae on its under surface. Halteres yellow.

Wing length. Specimens of this species from the South Indian hills are comparatively larger and the average length of the wings is 3.4 mm, while in specimens from Mahableshwar, Savantwadi Ghat, Mercara (Coorg) and Gersoppa it is only 2.6 mm.

The cluster of minute tubercles on the floor of the buccal cavity is like that in *himalayense*.

Spermatheca single, dark brown and globular.

MALE

Head black with a fringe of short blackish hairs on the occiput, face whitish grey, with scanty black hairs. Antennae black with very fine pale pubescence. Palpi black and of the usual form.

Thorax velvet black, covered uniformly and densely with short golden hairs. In the fore corners of the mesonotum are a pair of silvery spots, only half of each reflecting light at a time. In certain lights the sides and the hind margins of the mesonotum also show a broad silvery band. Pleurae dull ash grey, the membranous area bare.

Abdomen velvet black with lateral silvery spots on segments 2 and 5-7, those on segment 2 the brightest and connected with each other dorsally by a transverse grey spot along the anterior border of the segment. The basal scale is black with the fringe of long hairs nearly black. The abdomen has widely scattered short fine golden black pubescence on the dorsum and a number of long pale hairs arising ventro-laterally from segments 2-4. The genital armature resembles that of *himalayense*.

Legs show a great variation* in their colour and a certain amount in the relative size of the basitarsus of the hind leg. In the type male fore coxæ greyish pale yellow, trochanters yellowish grey, femora golden grey with a dark tip, tibiæ nearly black, with a large white spot on the outer side. Tarsi black moderately flattened. Middle coxæ black, trochanters and femora as in the fore leg, the latter without a black tip, tibiæ yellowish grey gradually darkened distally, tarsi dark grey (nearly black) except the basal half of the first segment which is slightly yellowish grey. Hind coxæ black, trochanters and femora slightly yellowish grey, tibiæ nearly black, slightly yellow basally. The tibiæ are comparatively narrower in the basal one-third than those in *himalayense*. Basitarsus yellowish in the basal half gradually becoming dark grey to nearly black distally, bases of second and third tarsal segment yellowish the rest nearly black. The basitarsus is moderately enlarged. It is a little shorter than the tibia and its greatest width is about 0.77† of that of the latter and nearly a quarter (0.26) of its own length.

Wings as in the female. Length 3.5 mm.

Besides specimens (of this species) bred out from other places, a large number of paratype specimens, along with a number of females, were bred from pupæ, collected from various streams near the Pasteur Institute at Coonoor from which locality the type female was collected by Senior-White.

The type male is for the present in my own collection.

PUPA (Text-figure 2)

Size about 3.3×1.5 mm.

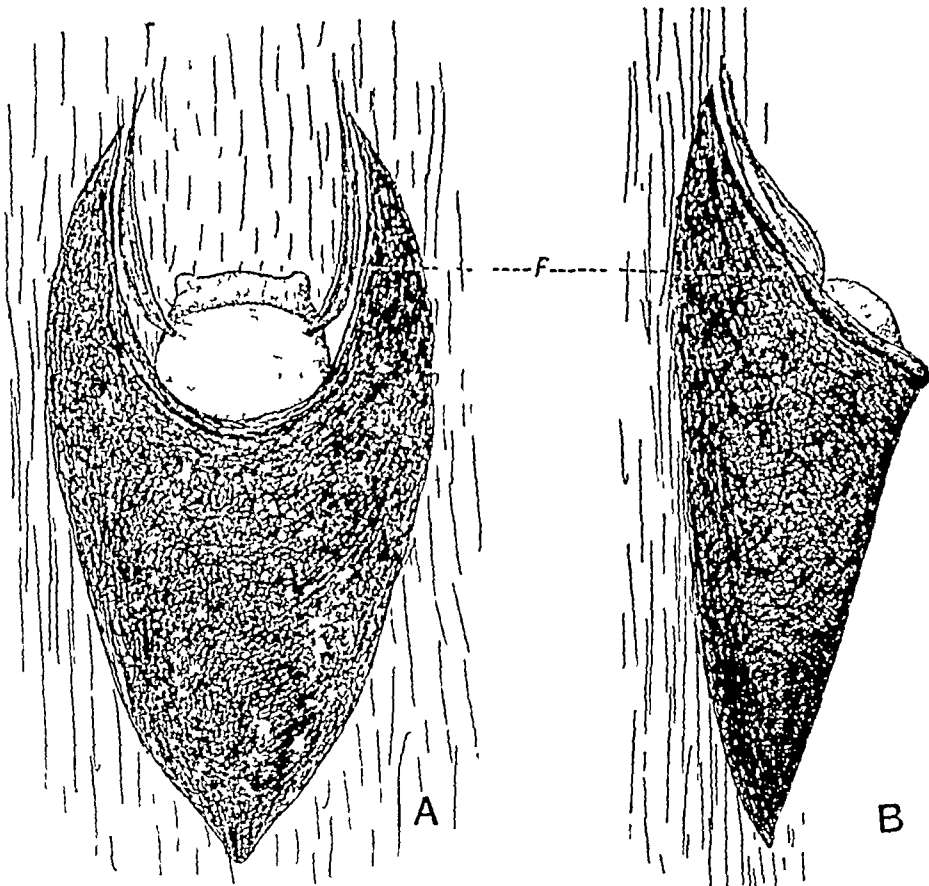
The integument of head and thorax brown to dark brown, the anterior two-thirds of the mesonotum free from chitinous tubercles, present in the pupa of *himalayense*, very minute tubercles being present only on the posterior one-fourth to one-third. Trichomes, hooks, rows of cuticular spines and the subterminal spines as in *himalayense*, except that a sensory hair on ventral surface of segment 4 is strongly chitinized and slightly bent forwards in this species.

Respiratory filaments (Plate L, fig. 9), about 2.6 mm long. Unlike those found in the other species, they are metallic in colour (dark grey to light grey).

* In the males of this species from Mahabaleshwar and in a few from the South Indian hills the trochanters, femora and the middle tibiæ are practically all deep yellow, the hind tibiæ and basitarsi also having more yellow on them than in the type specimen. Forms having intermediate coloration between the above and that found in the type male are also common among the specimens from Kodikanal. (Even in specimens with pale legs the fore and the hind tibiæ and tarsi have practically the same colour as in the type male.) Among the specimens from Kodikanal there is another form of male of this species. In these the legs are nearly all black, the only yellowish pigment present being on the front coxæ and the hind basitarsi both of which are like those in the type specimen. Intermediate forms between the all dark legs and like those in the type are also found but are not as common as between the pale and the type form.

† In some specimens from Kodikanal the hind basitarsus is comparatively broader while in some from Mahabaleshwar it is narrower than in the type but the intermediate forms are fairly common.

Six on each side, arranged in three pairs, the upper and the lower with very short stalks while the middle arises more or less from the main stem. The filaments decrease in thickness from above downwards, the uppermost being the stoutest of all, the upper filaments are directed forwards practically straight out at their origin and after a very short course bend downwards, resembling the arrangement found in the European species *S. monticola* Fried (Puri, 1925,



Text figure 2

Simulium gurneyae S W A and B —Dorsal and lateral views of pupa respectively in cocoon
(Lettering and scale as in Text figure 1)

p 343) The surface of the filaments is raised into very small tubercles which are somewhat uniformly distributed there being no definite ridges

Cocoon is dark brown, of the ordinary wall-pocket type (Text-figures 2 and 3), tough and compact without any windows. It nearly covers the whole pupa. Its length at the base is about 4 mm and at the dorsal end is 2.7 mm. Its greatest width about the middle is about 2.2 mm. The cocoon has a different shape to that found in *himalayense* and is comparatively much wider.

DISTRIBUTION

This species was originally described from Coonoor (25 ix 20) and I have bled out large numbers of specimens of this species from pupæ collected from the following places. From streams in and around Coonoor (6,000 ft) 28-31 xii 27, from a stream in Ootacamund (7,000 ft) 16 i 28, from Poona 21 xii 30, from small streams near Old and New Mahableshwar (4,500 ft) 23 xii 30, Streams crossing the ghat road between Savantwadi and Belgaum 29 xii 30, near Gersoppa (Bombay Presidency) 3 i 31, streams in Mercara (Coorg) 9 i 31 and streams in and around Kodikanal (Palm Hills) 24-25 i 31

Simulium (Simulium) nilgircum SP. N

FEMALE.

Head slate grey, with short black hairs on the occiput. Frons slate grey, somewhat shining, more or less rectangular, being not much narrowed in the region of the antennæ, a little more than half as broad as it is long and has a few dark hairs along the lateral borders. In the paratype specimen these hairs as well as those on the occiput are somewhat golden. Face with silver grey dusting, with scattered black hairs some of which are golden. Antennæ. The scape and the basal segment of the flagellum are yellowish grey, the rest dark (nearly black) with very fine pale pubescence. In the paratype specimen the scape and the basal flagellar segment are reddish in colour and the pubescence is somewhat golden. Palpi black and of the usual form.

Thorax dark, mesonotum only a trifle shining with greyish reflections and fine grey dusting in the anterior region. The grey dusting is in the form of a pair of rounded spots in the fore corners. When viewed from in front the mesonotum shows four greyish lines, two broad submedian separated from each other by a narrow black line and two narrow lines only a little external to the broad ones, all these grey lines end posteriorly before the middle of the mesonotum. The mesonotum is densely covered with coarse golden pubescence. Scutellum also with dense golden and black marginal hairs. Pleuræ with ash grey reflections, membranous area bare.

Abdomen blackish brown (nearly black), the fringe of long hairs on the dark brown basal scale pale golden. Second segment dark brown with a silvery spot dorsally, dorsum of segments 6-8 shining and sparsely covered with very fine pale hairs. Venter dark brown. Terminalia as in *himalayense*.

Legs. Front coxæ yellowish brown, trochanters and femora brownish, the latter gradually becoming darker distally, tibiae dark brown with a silvery white patch on the outer side, tarsi brownish black, moderately expanded, the first segment being six times as long as its greatest width at the distal end, segments 1, 3 and 5 with a pair of long black hairs apically on the outer side. Middle and hind coxæ nearly black, trochanters and femora dark brown, tibiae yellowish brown basally

gradually becoming dark brown in the distal two-thirds. In certain lights the bases of the tibiae with a whitish sheen on the posterior surface. Basal third of the first segment of the middle tarsi yellowish brown and in the hind tarsi the basal two-thirds of segment 1 pale yellow and the base of segment 2 yellowish brown, the rest of the tarsi are brownish black (dark brown). All coxae, trochanters, femora and middle and hind tibiae with a golden pubescence. Pedisulcus well marked, calcpala of moderate size, comparatively smaller than in the other two species. All claws with a small sub-basal tooth.

Wings normal hyaline, radius bare up to the fork. The radial sector simple, concave vein bearing setae on its under surface. Halteres pale yellow.

Wing length 3 mm

A number of small chitinous teeth-like tubercles form a cluster on the floor of the buccal cavity at its posterior end. These teeth are a trifle larger than those found in *himalayense* and *gurneyæ*.

Spermatheca single, dark brown and globular in form.

MALE

Head black with a fringe of short black hairs on the occiput. Face dusted with ash grey, with some black hairs. Antennae black, with very fine pale pubescence. Palpi black.

Thorax velvet black, covered with short golden hairs scanty on the middle of the mesonotum (most probably rubbed off). A pair of silvery spots in the fore corners of mesonotum, only one half of each reflecting light at a time. In certain lights the sides and the hind margins (prescutellar region) also show a broad silvery band. Pleurae with ash grey reflections, membranous area bare.

Abdomen velvet black with lateral silvery spots on segments 2 and 5-7, those on segment 2 brightest and connected with each other dorsally by a transverse grey spot along the anterior border of the segment. The basal scale is brownish black with the fringe of long hairs black with golden reflections. On the dorsum are scattered short golden black hairs and some long pale ones arise latero-ventrally from segments 2-4. Venter dull brownish. The genital armature is like that of *himalayense*, the styles, however, appear to be somewhat larger comparatively and the projecting lip of the inter-coxal piece also is comparatively larger.

Legs predominantly black. Front coxae brownish black, posterior black, all trochanters and femora brownish black, the hind femora darker than the anterior ones. Front tibiae black with a whitish spot on the outer side, tarsi black, only slightly flattened, being nearly 8 times as long as its greatest breadth at the distal end. Middle tibiae and tarsi brownish black, hind tibiae black, basal half of basitarsus yellowish brown, the rest of tarsi brownish black. Basitarsus of the hind leg moderately enlarged, a little shorter than the tibiae and its greatest width (about the middle) is about 0.77 of the greatest width of the latter and nearly a quarter

its own length. All the legs have a fair amount of golden pubescence on coxæ, trochanters, femora and tibiae.

Wing as in the female, length 3 mm.

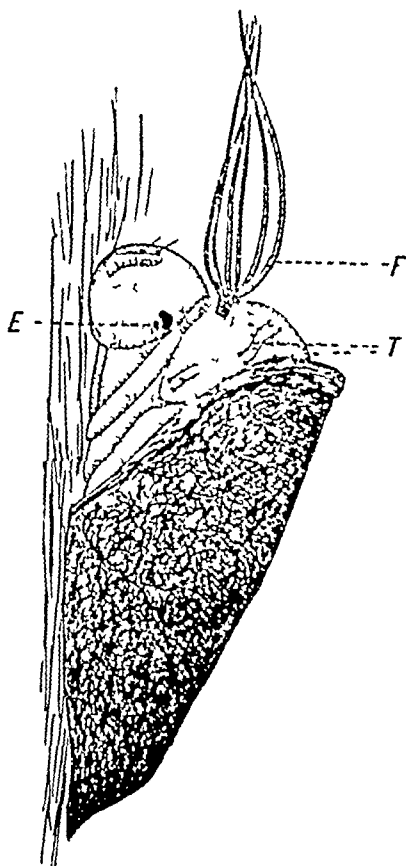
The male very closely resembles the very dark form of the male of *gurneyæ* from Kodikanal from which it only differs in having a larger number of golden hairs on the legs than in the latter species.

Described from three females and six males all bred out from pupæ collected at Coonoor, 28-31 XII 27.

Types and paratypes in my own collection.

PUPA (Text-figure 3)

Size about 3.2×1.1 mm.



Text figure 3

Simulium nilgiricum sp. n. Lateral view of pupa in cocoon

E —Remains of larval eye, *F* —Respiratory filaments, *T* —Thoracic trichomes

(Scale as in Text-figure 1)

Integument of head and thorax brownish, sparsely covered with chitinous tubercles which are comparatively larger than in *himalayense*. Trichomes, hooks

and rows of cuticular spines and subterminal spines as in *himalayense*, except that a pair of strong bifid spines curved forward is present on segment 4 ventrally, and a sensory hair lying near it is also slightly thickened

Respiratory filaments (Plate L, fig 10) are about two-thirds the length of the pupa, dull black in colour, 6 on each side, arranged in three pairs—the upper and the lower of which have very short stalks and the middle one arises more or less directly from the main stem. All the filaments are more or less of the same thickness and spread out slightly from their origin. The surface of the filaments (Plate L, fig 11) is raised into minute tubercles which are of two kinds, the larger ones form a reticular pattern the interspaces being covered by the smaller tubercles. The larger tubercles take the place of the ridges (folds) found on the filaments of *himalayense*.

Cocoon is of the ordinary wall-pocket type but the anterior border forming the open end instead of being directed forward, as usual, is curved backward (Text-figure 3) so that the length of the cocoon at its base is much less than at the top. Length at the top is about 2.7 mm while that at the base is only about 1.8 mm (cf cocoon of *himalayense* and *gurneyæ*). The width at the anterior end is about 1.2 mm. The cocoon is pale dirty brown, loosely woven but without any broad spaces or windows in the mesh. It does not cover the pupa completely.

SUMMARY

Males, females and pupæ of two new species, one from the Himalayas and the other from the Nilgiri hills, have been described.

Male and pupa of *S. gurneyæ* Senior-White have been described for the first time and a revised description of the female has been given.

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EXPLANATION OF PLATE L

Simulium himalayense sp. n.

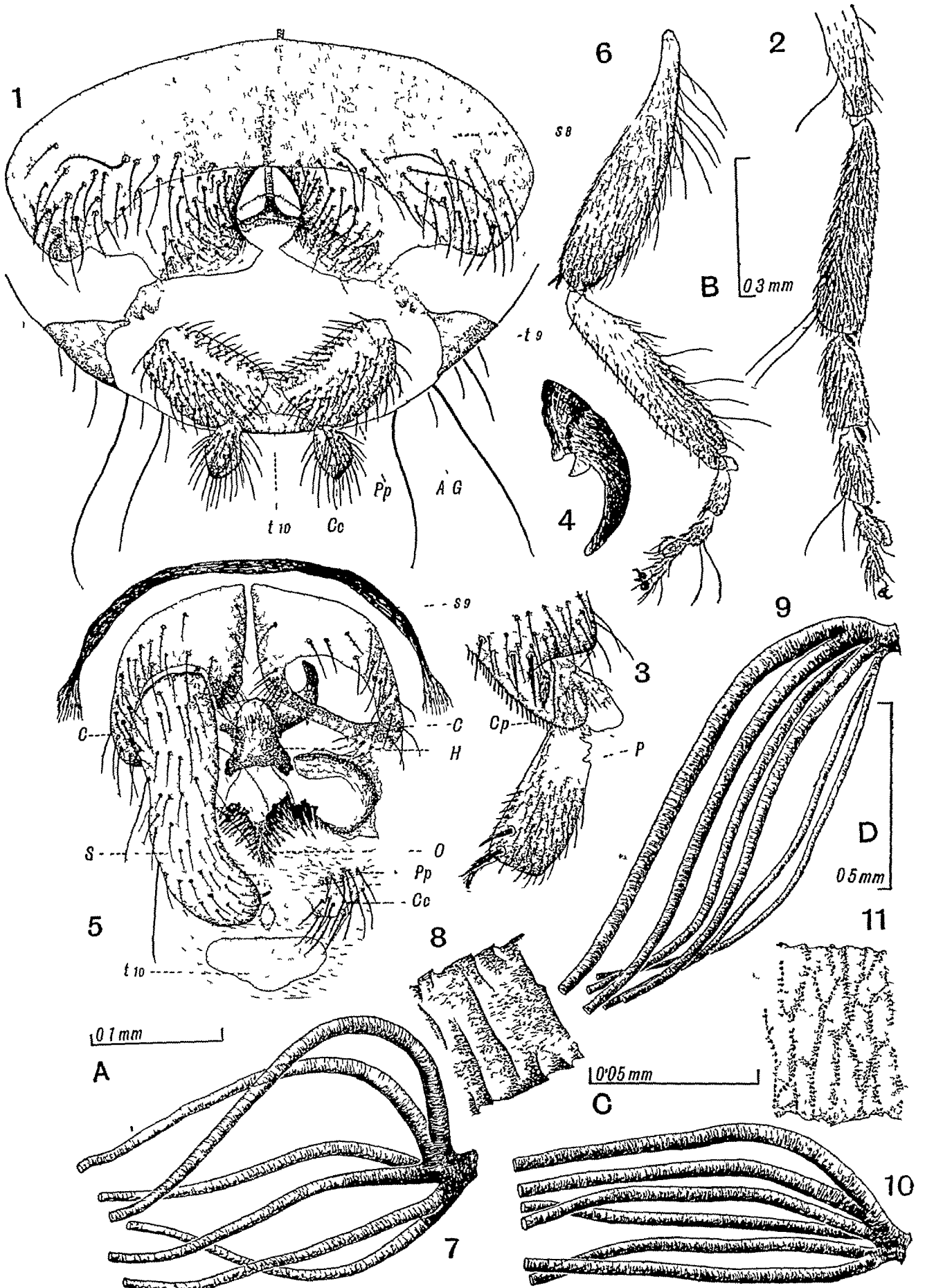
- Fig 1 Ventral view of the posterior end of a paratype female Scale A
 , 2 Tarsal segments of left front leg of a paratype female Scale B
 „ 3 Part of basitarsus and of second tarsal segment of left hind leg of a paratype male, showing calcipala and pedisulcus Scale A
 „ 4 A claw from hind leg of a paratype female Scale C
 5 Ventral view of posterior end of a paratype male Left style removed, only one cercus shown Scale A
 , 6 Tibia and tarsus of left hind leg of a paratype male Scale D
 „ 7 Respiratory filaments of left side of pupa Scale D
 „ 8 Part of the surface of respiratory filaments enlarged Scale C
 „ 9 *Simulium gurneyi* S. W. Respiratory filaments of left side of pupa Scale D

Simulium nilgircum sp. n.

- „ 10 Respiratory filaments of left side of pupa Scale D
 „ 11 Part of surface of a respiratory filament enlarged Scale C

A G—anterior gonopophyses, *C*—coxites, *Cc*—Cerci, *Cp*—Calcipala, *H*—Intercoxal piece, *O*—Genital opening, *P*—pedisulcus, *Pp*—paraprocts, *S*—style, *s8* and *s9*—8th and 9th sternites respectively, *t9* and *t10*—9th and 10th tergites respectively

PLATE I



STUDIES ON INDIAN SIMULIIDÆ

Part II.

DESCRIPTIONS OF MALES, FEMALES AND PUPÆ OF *SIMULIUM* *RUFIBASIS* BRUNETTI, ITS VARIETY *FASCIATUM* NOV VAR AND OF THREE NEW SPECIES FROM THE HIMALAYAS

BY

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[Received for publication, October 10, 1931]

THE four species described in this paper, like those dealt with in Part I, belong to the subgenus *Simulium*, having basal section of radius bare, front tarsi flattened, claws of female without a basal tooth and the tergites of abdominal segments 6-8 shining. They are all characterized by having a more or less shining frons in the female and 6 respiratory filaments in the pupal stage.

Except the species *S. ramosum*, which has been based on the distinctive structure of the pupa the other three can be easily separated from one another and from the three species, described in Part I, of this study, on the character of the terminalia of their males and females. It has been observed that the terminalia of the female are as useful for the identification of the different species, as those of the male, which only are usually described by most people working on this family.

***Simulium (Simulium) rufibasis* BRUNETTI 1911**

S. rufibasis was described by Brunetti (1911) from a single female collected at Kurseong (Darjeeling District, N E Bengal) by Dr Annandale (18 vi 10). I have since bred out from pupæ a large number of males and females of two distinct species the adults of which resemble each other very closely and the description

of the female given by Brunetti applies more or less to the females of both of them. It would have been difficult to decide definitely as to which of them was *rufibasis* but, fortunately, whereas both the species occur together in the hills around Simla only one has so far been collected from Kurseong and its vicinity. Consequently there is no doubt as to the correct identification of the species herein described as *rufibasis*, and complete descriptions of the female, as well as of the male and pupa of which are given below.

FEMALE

Head greyish black with short slender dark hairs on the occiput, present though scanty on the face and a few also on the lateral borders of the frons. Frons distinctly shining, greyish black, in certain lights appearing black, with nearly parallel sides, about one and a half times as long as its greatest width at the top. Face dull ash grey. In some specimens from Kurseong and in a number from other localities it is comparatively lighter than in the type female. *Antenna* In the type specimen the scape and two basal flagellar segments are deep orange and the rest is brownish black with a fine pale greyish pubescence. The colour of the three basal segments is, however, very variable, varying from pale yellow to greyish yellow and orange to reddish brown. In some specimens only the proximal halves of the three basal segments are reddish brown and the rest nearly black. Palpi reddish brown to black.

Thorax Mesonotum black, somewhat shining but when viewed from in front it is dull grey up to prescutellar area which is black. In some specimens this greyiness is more marked in the anterior region in the form of a pair of large rounded silvery spots. Mesonotum sparsely covered with brassy pubescence, partly rubbed off in the type. Scutellum slightly reddish brown in type but varying in colour from dark brown to black, with black marginal hairs and a few golden hairs* scattered along the margin. When viewed from in front the scutellum appears greyish in some specimens. Pleuræ and sterna dull, slate grey, membranous area of pleuræ bare.

Abdomen brownish black, second segments with light greyish dusting. The fringe of hairs of the basal scale vary in colour from black to slightly golden. Tergites of segments 6-8 shining, with scattered short and slender pale hairs, some present also on the anterior segments. *Terminalia* (Plate LI fig 1) The ventral surface of segment 7 bears a pair of submedian clusters of fairly long and thick black setæ (Plate LI, fig 2). Sternite of segment 8 is comparatively narrow with more or less pointed lateral ends and bears only a few long hairs antero-laterally. Anterior gonopophyses with only a few short hairs near their anterior borders, their inner border is not thickened and is more or less straight. Paraprocts are

* Rubbed off in the type specimen

thinly chitinized, except their interno-anterior portion which is comparatively thicker and bears comparatively finer setæ, cerci are of moderate size

Legs Fore coxæ yellow, trochanters brownish yellow, femora dark brown, somewhat pale at the basal end but nearly black distally, tibiæ pale yellow, the distal one-third (to one-fourth) black, the outer side with a large silvery white patch, tarsi black, moderately expanded, first segment a little less than five times its greatest width at the distal end, and segments 1 and 2 together a little shorter than the tibiæ, segments 1 and 3 with a pair of long black hairs subapically on their posterior border Middle and hind coxæ black, trochanters dark brown, femora dark brown nearly black distally, tibiæ pale yellow in the basal two-fifths to one-third, gradually becoming black distally with a large silvery patch on the posterior surface of the basal half, tarsi a little longer than the respective tibiæ, basal one-third and basal half to one-third of the first tarsal segment of the middle and hind tarsi respectively, pale yellow, the rest black* Femora, tibiæ and pale parts of the tarsi with sparse fine, brassy, pubescence Pedisulcus well marked, calcpala of moderate size, extending up to pedisulcus All claws simple (Plate LI, fig 3)

Wings hyaline, radius bare up to the fork, radial sector simple, concave vein bearing setæ on its under surface Halteres pale to orange yellow, stalk dark basally

Average wing length 2.5 mm

The ventral surface of the buccal cavity at its posterior end bears a cluster of minute nodules

The furca is slightly expanded at its anterior end and the spermatheca is single, globular in form and uniformly dark brown

MALE.

Head black with a fringe of short dark hairs on the occiput Face whitish grey, having a whitish sheen in certain lights and with scattered black hairs Antennæ black with very fine whitish pubescence Palpi black

Thorax Mesonotum velvet black, covered uniformly but sparsely with fine, copper coloured pubescence, and also long scattered black hairs on the prescutellar region Anteriorly are a pair of elongated silvery spots broadly separated in the middle These spots have a somewhat metallic sheen and are seen more or less complete In certain lights the mesonotum shows a broad silvery border laterally and posteriorly, connected in front to the anterior spots Scutellum is black with only a few fine copper coloured hairs and a fringe of long black hairs Pleuræ with a silvery sheen, membranous area bare

* Out of about 30 specimens two had even darker legs

Abdomen velvet black, with a few fine hairs like those on the mesonotum. Usual silvery spots present on segments 2 and 5-7. Long hairs on the basal scale nearly black. *Genital armature* (Plate LI, fig 4) Somewhat resembles that of *S. himalayense* (Puri, 1932). Coxites short, a little shorter than their breadth, styles long, about three times as long as their breadth near the base. They are slightly wider a little beyond their base and in this region on their dorsal surface they bear a cluster of well-developed denticles. Distally the styles are a little narrower than in the proximal half. Each bears a single short spine subterminally on the inner edge. The inter-coxal piece has a short moderately broad base from which a broad tongue-like process projects downwards and curves forwards. This process is densely covered all over with fine conspicuous setae.

Legs are black, except for a large silvery white patch on the outer side of fore tibiae and a slight paleness at the base of the middle and hind tibiae on their posterior surface. The base of the 1st tarsal segment of middle leg and the basal one-third or so of the basitarsus of the hind leg are also slightly yellowish. Fore tarsi are moderately expanded, the first segment is a little shorter than six times its greatest breadth at its distal end. The basitarsus of the hind leg (Plate LI, fig 5) is much enlarged, its length is 0.8 of that of the tibia and it is a little wider than the greatest width of the latter and 0.36 its own length.

Wings as in female.

A number of paratype specimens along with a number of females of this species were bred out from pupae collected from various streams in and around Kurseong from which locality the type female was originally described. The type male is for the present in my own collection.

PUPA

Size about 2.5×1.0 mm.

The integument of the head and thorax brownish, covered with moderate sized disc-like tubercles. Trichomes (Plate LI, fig 6), hooks, rows of cuticular spines and the subterminal spines as in *himalayense*, except that on the ventral surface of segment 4 there is a pair of strong bifid spines curved forward and the subterminal spines are comparatively poorly developed.

Respiratory filaments about 2.0 mm long, light grey, somewhat metallic, 6 on each side, arranged in three pairs, the upper and the lower with a short stalk while the middle one may have a short stalk or may arise more or less directly from the main stem. The uppermost filament is directed forwards and upwards and after a short course bends downwards more or less at right angles. All the filaments run practically parallel to one another (Plate LI, fig 7). The uppermost filament is the stoutest of all which decrease slightly in thickness from above downwards. The arrangement of the ridges and tubercles on the surface of the filaments resembles that found in *himalayense*.

Cocoon is light brown, of the ordinary wall-pocket type resembling that of *himalayense*. It is fairly compact without any windows or spaces in the mesh. It nearly covers the pupa. Its length at the base is about 3.0 mm, while at the top it is 2.5 mm and its greatest width at its anterior end is 1.4 mm.

DISTRIBUTION

I have reared specimens of this species from pupæ collected from Balasan River and other streams near Marianbarie, Bengal Tarai (March 1927), various streams in and around Kurseong (August 1927), small streams crossing the Kalka-Simla Motor Road (near Kandaghat) (27 viii 29), streams around Simla (stream below Annandale, about 6,000 ft, 3 vii 26, stream north of Simla E about 6,000 ft, 6 ix 30, Chadwick Falls, about 5,500 ft, 7 ix 30) breeding together with *himalayense*, from large torrential streams crossing the Hindustan-Tibet Road (mile 30/31 from Simla) about 8,200 ft, 11 ix 30, in conjunction with a number of other species.

Simulium rufibasis var *fasciatum* NOV. VAR.

Among the specimens of *S. rufibasis* bred out from pupæ collected from parts of the Western Himalayas a certain number of males and females appear a trifle larger in size and, although hatching out of pupæ identical to those of the type, show a certain amount of difference, in their ornamentation, from that of the type species. The differences between the males are so marked and constant that at first sight they indicate a separate species but in view of the fact that the pupæ and their cocoons do not show even the slightest difference and that the differences between the females too are very slight it has been thought advisable to describe these specimens as only a variety of *rufibasis*.

FEMALE

The female resembles that of the type very closely differing from the latter only in having a trifle paler legs. The thorax appears to be comparatively duller and when viewed at a certain angle from behind it shows a pair of very small silvery grey spots near the anterior border of the mesonotum. These spots are not very clear in the type species.

Legs Fore coxæ and trochanters pale yellow, femora brownish yellow in the basal one-third, gradually becoming brown to black distally, tibiæ yellow, distal one-fourth to one-fifth black with a large silvery spot on the outer side, tarsi black, moderately expanded, first segment about five times its greatest width. Middle and hind coxæ black, trochanters greyish yellow, femora black, with pale bases, the basal two-thirds and a little more than the basal half of the middle and hind tibiæ respectively pale yellow, the rest becoming black gradually. Basal two-thirds of the first and the bases of the second tarsal segments yellow, the rest

of tarsi black The legs have comparatively more golden pubescence than in the type species

Wing length about 2.9 mm

MALE

The males differ from those of the type species in their size and the ornamentation of the thorax and the legs, resembling the latter in all other respects

Thorax is black but the velvety appearance is not well marked Mesonotum covered uniformly and fairly densely with short golden hairs which are comparatively much coarser than in the type species When seen from in front the mesonotum shows a pair of large silvery spots which are not seen complete and three narrow silvery stripes running longitudinally, one median and two submedian, the latter meeting the inner borders of the rounded spots anteriorly All the three lines are connected with each other by a transverse line just in front of the prescutellar region As the specimen is slowly turned and the angle of vision changes the large rounded spots and the lines disappear and the part of the large spots not visible previously is now seen as a rounded spot at the anterior border of the mesonotum The mesonotum shows a broad silvery border laterally and posteriorly, this band being connected to the rounded spots anteriorly In certain lights, in some paratype specimens, the area between the three lines and the anterior half of the mesonotum has a silvery sheen and in others the longitudinal strips are not well marked but the silvery greyiness of the anterior half is only present

Legs Fore coxae yellow, posterior ones black, all trochanters dark grey with yellow bases, all femora black with yellowish bases Fore tibiae yellow in basal two-thirds, the rest black, the outer surface with a large silvery white spot, tarsi black moderately flattened, the first segment about six times as long as its greatest breadth at the distal end Middle and hind tibiae yellow in the basal half, black distally, with a silvery sheen basally on the posterior surface, basal one-third of the 1st and the base of the 2nd tarsal segments yellow, the rest of tarsi black The basitarsus of the hind leg is much flattened It is a trifle shorter than the tibia and a little broader than the latter, about 0.38 of its own length * Out of 13 male specimens of this variety in one the tibiae are comparatively darker and only a small portion of the posterior two pairs is yellow

Length of wing 2.8 mm

Described from 17 females and 13 males all bred out of pupae collected from Simla (small stream East of Chhota Simla 6,000 ft, 6 ix 30, Chadwick falls, 7 ix 30), a number of streams crossing the Hindustan-Tibet Road between

* The legs in this variety have the appearance of being banded

Theog and Narkanda, 7,500–8,500 ft and from a fast hill stream at Naranag (Kashmir), September 1930, the last by Colonel Sir S R Christophers

Types and paratypes for the present in my own collection

Simulium (Simulium) ramosum SP N

The adults of this species resemble those of *S. rufibasis* so closely that, in spite of a minute and careful study, I have not been able to find any constant difference between them, but the difference in the pupæ are so marked that I have no hesitation in treating them as belonging to two distinct species. Only slight differences are found between the males. Some paratype females have a little paler legs than in the type specimen and resemble the variety *fasciatum*.

MALE

In the male the pubescence of the thorax resembles that found in *fasciatum*. The silvery grey spots are large and only half of them is visible at a time, the longitudinal stripes are absent though in some specimens a slight silver grey dusting is seen on the anterior portion of the mesonotum between the two rounded spots.

Genital armature resembles that of *rufibasis*, the styles, however, appear comparatively a trifle broader distally and the chitinous teeth forming a cluster on the dorso-internal surface about the middle are somewhat bigger but this has been seen even in some specimens of the variety *fasciatum* also.

Legs are black, excepting the following portions, coxæ, trochanters and bases of femora of front legs are yellowish, basal half of the basitarsus and the base of the 2nd tarsal segment of the hind leg are yellow. Besides the large silvery spot on the outer side of the fore tibiæ, the bases of the middle and hind tibiæ also have a whitish sheen on the posterior surface. Unlike the hind basitarsus of *rufibasis* male only the basal third of which is diffusedly yellowish, in this species the yellow and black halves are well marked and the yellow colour is not mixed up with the black. The hind basitarsus appears comparatively less broad. It is a little shorter than the tibiæ and about as wide as the latter and 0.35 its own length. It also seems comparatively less wide in its proximal portion than the hind basitarsus in *rufibasis*.

Wing length is about 2.9 mm.

Described from 17 males and 16 females all bred out of isolated pupæ collected from various streams in the Simla hills breeding together with *rufibasis* and *himalayense*.

PUPA

Size about 3.0 × 1.4 mm.

The integument of the head and thorax brown to dark brown covered with flattened chitinous tubercles, those covering the anterior half or two-thirds of the

mesonotum (Plate LI, fig 8) are comparatively larger than those in *rufibasis*. Cephalic and thoracic trichomes moderately long, and branched, those in the submedian group splitting near their base into 4 to 7 diverging branches. On the abdomen hooks, rows of cuticular spines and subterminal spines as in *rufibasis*, a pair of strong bifid spine being present on the ventral surface of segment 4.

Respiratory filaments—Their average length is 2.2 mm, about 0.7 of the length of pupa, somewhat dull dark grey in colour, 6 on each side arranged in three pairs, the upper and the lower of which have a short stalk while the middle one appears to arise from the somewhat dilated stem of the upper pair (Plate LI, fig 9). The bases of the upper four filaments are slightly swollen. The filaments curve downwards from their origin and run parallel to the border of the cocoon. The surface of the filaments shows a similar structure as seen in *S. nilgircum* but in some specimens ridges too are to be seen.

Cocoon is dirty brown, of the ordinary wall-pocket type, tough and compact without any space or windows in the mesh. It has a fairly strong anterior rim and nearly covers the pupa. Its length at the base is 3.3 mm and at the dorsal end is 2.5 mm, its greatest width near the anterior end is about 1.7 mm.

***Simulium (Simulium) christophersi* SP. N.**

FEMALE

Head black, with fairly dense short black hairs on the occiput. Frons dark grey, somewhat shining, a trifle narrowed in the region of the antennae a little longer than its greatest breadth near the top, with sparse slender dark hairs on its lateral and ventral portions. Face dusted with whitish grey, with scanty short black hairs. Antennae with scape and basal flagellar segment dark brown the rest black, with a fine whitish pubescence. In some paratype specimens antennae are completely black. Palpi black.

Thorax Mesonotum covered with pale golden pubescence, when viewed from in front it is greyish with a narrow median and two broader submedian black lines forming a lyre-shaped mark, the submedian lines are continued laterally along the anterior border and are connected to a black stripe running along the lateral border, when viewed from behind the colours are as usual reversed, the lateral stripes and the portion connecting it anteriorly to the submedian lines become conspicuously silvery. Scutellum black with golden pubescence and black marginal hairs. Pleurae dark grey membranous area bare.

Abdomen black, practically bare long hairs on basal scale golden, second tergite with silvery grey dusting. Tergites of segments 6–8 very large and shining with some short black hairs. Venter pale brownish. *Terminalia* (Plate LII, fig 10). Hairs on the ventral surface of segment 7 uniformly distributed. Sternite of segment 8 with drawn out narrow lateral ends, posterior border sharply

rounded, comparatively short and fine hairs on the lateral portions, the middle third free from hairs. Anterior gonopophyses with a number of rather short hairs scattered all over, their interno-lateral border slightly curved and thickened. Paraprocts poorly chitinized and the cerci comparatively small.

Legs Fore coxæ and trochanters pale yellow, the latter dark grey distally. femora yellowish with the tip yellowish black, tibiæ pale yellow, the distal one-fifth nearly black, the outer side with a large whitish patch which is not conspicuously silvery, tarsi black only slightly flattened, the first segment about six times as long as its greatest width at the distal end. Segments 1 and 3 with the usual pair of long black hairs subterminally on their posterior border. Middle and hind coxæ black, trochanters yellowish with dark distal ends. Middle femora dirty pale yellow with a black tip, tibiæ yellowish gradually becoming dark in the distal one-third, the posterior surface with a whitish sheen. Base of the first segment slightly yellowish, the rest of the middle tarsi black. Hind femora and tibiæ dirty yellow, gradually becoming black in the distal one-fourth,* the latter with a whitish sheen on the posterior surface of its basal half, first tarsal segment yellowish, gradually becoming black in the distal one-third and with its anterior border black, basal half of second segment yellowish black, the rest of the tarsus black. All yellow parts of the legs with fine pale golden pubescence. Pedisulcus well marked, calcpala of moderate size. All claws comparatively slender, and with a poorly developed sub-basal tooth (Plate LII, fig. 11).

Wings Normal hyaline, radius bare up to the fork, radial sector a concave vein, with fine hairs on the ventral surface. Halteres yellow.

Wing length about 3.2 mm.

At the posterior end of the buccal cavity is a cluster of very short upright conical processes arising from the ventral surface, in place of the cluster of minute nodules present in the other species described in this paper.

Anterior end of the furca is not flattened and the posterior limbs are widely separated. Spermatheca single, globular, and uniformly dark brown.

MALE

Head black with a fringe of short black hairs on the occiput. Face, whitish grey, sparsely covered with black hairs. Antennæ black with a very fine whitish pubescence. Palpi black.

Thorax Mesonotum velvet black, covered uniformly and fairly densely with short golden hairs. In the type specimen the velvety sheen of part of the mesonotum has been rubbed off and such parts have become shiny and some of the golden hairs have also been rubbed off. Anteriorly are a pair of elongated silvery spots broadly separated in the middle. The spots are seen whole and not only

* In some paratype specimens there is more black pigment on the legs.

half at a time. In certain lights the mesonotum shows a narrow silvery border laterally and an inconspicuous one posteriorly. The lateral band is continuous with the anterior spots. Scutellum black, covered with golden hairs and has a fringe of long black hairs. Membranous area of pleuræ bare.

Abdomen velvet black, with scattered short golden hairs on the dorsum and a small cluster of long black hairs arising ventro-laterally from segments 2-4. Silvery lateral spots as usual on segments 2 and 5-7. Long hairs on the basal scale black. *Genital armature* (Plate LII, fig. 12), to some extent resembles that of *S. himalayense*. The coxites are short, about as broad as they are long, styles are comparatively long, nearly three times (or a little more than three times) as long as their breadth near the base, of more or less the same width throughout their length. On their dorsal surface in the proximal one-third they bear a small protuberance bearing short fairly thick close-set setæ. The single subterminal spine on the inner edge of each of the styles is comparatively very short. The inter-coxal piece has a moderately broad base from which a slightly flattened keel-like process with two rows of strongly chitinized teeth on its free edge projects downwards. This process is continued forward as a narrow somewhat thinly chitinized projection bearing fine backwardly directed setæ along its lateral borders. The anterior chitinization of the mesosome just behind the coxites, are comparatively much broader than in *himalayense*.

Legs. Fore coxæ brownish yellow, posterior ones black. All trochanters, femora and tibiae brownish black with their distal ends black, the middle comparatively lighter in the basal half than the others. Fore tibiae with a large whitish spot on its outer surface and the middle and hind ones with a faint whitish sheen on their posterior surface basally. Fore tarsi black very little flattened, being nearly 8 times as long as their greatest breadth at the distal end. The basal half of the hind basitarsus greyish black, the rest of tarsi black. The hind basitarsus moderately enlarged (Plate LII, fig. 13). It is a little shorter than the tibiae (0.74 of the length of latter), about as broad as the latter and about one-third its own length. The legs bear scattered golden pubescence which is not very conspicuous.

Wing as in female.

PUPA

Size about 3.0×1.1 mm

The integument of the head and thorax brown to dark brown, smooth and shining, free from any disc-like tubercles, except in the region of the metanotum where there are a few very minute ones. Head and thoracic trichomes as in *himalayense*, simple, moderately long. Dorsal and ventral hooks on the abdomen and subterminal spines as in *himalayense*. Only in exceptional cases is a sensory hair slightly thickened on the ventral surface of segment 4. Dorsally along the anterior border of segment 8 are a pair of submedian groups of backwardly directed

cuticular spines arranged in a row, a few minute spines being present in a similar position on segment 7 and still smaller ones on segment 9

Respiratory filaments (Plate LII, fig 14) —About two-thirds the length of the pupa. They are whitish, 6 on each side, arranged in 3 pairs, the upper and the lower with short stalks while the middle usually arises from the main stalk. The main stem is directed straight out from the pupa and the filaments bend downwards from their origin (as shown in Plate LII, fig 14) and run more or less along the border of the cocoon. The surface of the filaments is raised into tubercles of two sizes, the large ones form a reticular pattern and the smaller ones fill up the interspaces, resembling the arrangement found in *S. nilgircum*. There are no ridges or folds on the filaments as found on those of *himalayense*.

Cocoon is of the ordinary wall-pocket type, closely woven without any spaces or windows, thin but compact and somewhat shining. Its anterior border is slightly thickened. The size of the cocoon appears to be different in the male and the female, that of the former being a trifle smaller. The average length of the cocoon of a female pupa at the base is 3.6 and at the top 3.0 mm, while that of a male pupa at the base is 3.2 mm and at the top 2.8 mm. The average width of the cocoon is 1.4 mm. The cocoon covers the pupa completely.

Described from 29 males and 38 females all in good condition and bred out of isolated pupæ.

Types and paratypes for the present in my own collection.

I have great pleasure in naming this species *S. christophersi* after Colonel Sir Samuel Rickard Christophers, who bred specimens of it from pupæ collected from a torrential stream at Nara-Nag, Kashmir (about 7,500 ft above sea level), September 1930. I have found this species breeding in very large numbers occurring together with *S. rufibasis* and *ramosum* in various streams crossing the Hindustan-Tibet Road between Matiana and Narkanda (8,000 ft–9,000 ft). In some places big boulders, over which water was flowing, were completely covered with innumerable larvæ and pupæ of this species (September 1930).

Simulium (Simulium) nitidithorax SP. N

FEMALE

Head black, with scanty fine black hairs on the occiput and the face, a few along the lateral border on the frons also. Frons black, markedly shining, rather narrow, a little less than twice as long as it is broad about the middle, a little narrowed just above the region of the antennæ. Face also shining, greyish black. Antennæ with scape and two basal flagellar segments yellow, the rest yellowish black, covered with a fine pale pubescence. The colour of the scape and two basal

segments varies in the different paratype specimens from yellowish to orange and reddish brown and the rest of the antenna from yellowish or brownish black to black. In some only the scape and one basal segment are orange, the rest of the antenna being black. Palpi black.

Thorax Mesonotum greyish black, markedly shining, with a slight metallic tinge, sparsely covered with very fine short black hairs. Scutellum also black with black marginal hairs, present also on the prescutellar area. Pleurae dull greyish black, membranous area bare.

Abdomen nearly black, practically bare, fringe of hairs on the basal scale fairly short, pale golden. Second tergite with the usual greyish reflections, tergites of segments 6-8 very large, slightly brownish black and shining, those of 6 and 7 with scattered very short pale hairs while 8th with black, comparatively longer hairs. *Terminalia* resembles those of *rufibasis* very closely but the short hairs on the ventral surface of segment 7 are scattered and scanty and the pair of clusters present in the latter species are absent. Steinite of segment 8 is ribbon-like with slightly narrow, rounded lateral ends as in *rufibasis*, its middle half free from hairs, a few stout ones present only on the lateral portions. Anterior gonopophysis well developed, free from long stout macrosetae, if present only a few short slender ones near their anterior border. Inner border comparatively slightly thickened and a little concave leaving a narrow space between them.

Legs Fore coxae yellow, trochanters yellow, dark distally, femora yellow in the basal half becoming blackish distally, tibiae dark, nearly black in the distal half, with large silvery white patch on the outer surface, tarsi black, moderately expanded, first segment about 5 times as long as it is broad at its distal end. Segments 1 and 3 with a pair of long black hair subapically on their posterior surface. Middle and hind coxae black. Middle trochanter yellowish brown, femora and tibiae brownish black, the latter with a silvery sheen basally on the posterior surface. Hind trochanters yellow, femora and tibiae brownish black with the bases yellowish, the latter darker than femora and with a silvery white sheen basally on its posterior surface. Basal three-fourths of the 1st and the basal half of the 2nd segment of the middle and hind tarsi yellow, the rest black. Calcipecta moderately large extending up to pedisulcus which is well marked. All claws simple.

Wing Normal hyaline, radius bare up to the fork, radial sector a concave vein bearing setae only on its ventral surface. Wing length 2.3 mm (average length in the paratype specimens is 2.2 mm). Halteres pale yellow.

Nodules forming a cluster on the ventral surface at the posterior end of the buccal cavity are very minute.

Furca of the usual form, its anterior end not much flattened. Spermatheca single globular, uniformly dark brown.

MALE

Head black, with a fringe of short pale hairs on the occiput. Face dusted with whitish grey, with scanty dark hairs.

Thorax Mesonotum velvet black, with scattered very fine copper coloured pubescence mostly rubbed off in the type. Setae from part of the mesonotum rubbed off giving that part a shiny appearance. Anteriorly are a pair of large silvery spots which are seen complete, a broad silvery border present laterally and posteriorly, connected anteriorly to the large spots. Scutellum black, with black marginal hairs. Pleurae nearly black with a silvery grey sheen, membranous area bare.

Abdomen velvet black, with scattered very fine pale pubescence, fringe of long hairs on basal scale brownish, segments 2 and 5-7 with the usual silvery spots. *Genital armature* (Plate LII, fig. 15) somewhat resembles that of *rufibasis*. Coxites about as long as broad, but appear comparatively longer than in *rufibasis*. The styles are rather long, commencing rather narrow at the base, they broaden out in the basal one-third and then again become narrow distally. They are about three and a half times as long as they are broad at the base. On the interno-dorsal surface of the region of their greatest width each of them bears a protuberance directed inwards and upwards towards the base. This protuberance bears strong well-developed teeth on its anterior free surface. The inter-coxal piece and the mesosome are like those found in *rufibasis*, but the former is a trifle narrower comparatively and has a longer base.

Legs Fore coxae yellow, trochanter and femora yellowish brown, the latter black distally, tibiae and tarsi black, the former with a large silvery spot on its outer surface. Tarsi flattened as in female. Middle and hind coxae black, trochanters yellowish brown basally, femora and tibiae black, the latter with a slight silvery sheen basally on their posterior surface. Basal two-thirds of the first and base of the second segment of the middle tarsi and the basal halves of the first and second segments of hind tarsi yellow, the rest black. Basitarsus of the hind leg only a little enlarged (Plate LII, fig. 16). It is about three-fourths as long as the tibia, and its greatest width is 0.7 of that of the latter and a trifle more than one-fourth its own length.

Wings as in the female.

PUPA

Average size 2.6×0.9 mm.

The integument of head and thorax brown to light brown, with very minute disc-like tubercles scattered all over, a trifle larger on the dorsum of mesonotum than on other parts. Head and thoracic trichomes as in *rufibasis* but only about one-third to one-fourth as long as those in the latter species. Hooks as in *himalayense*, except that in this species there is on the ventral surface of segment 4 a

pair of strongly chitimized bifid or simple spines bent forwards. Dorsally on segments 7, 8 and 9 there is a row of backwardly directed cuticular spines along the anterior border but none on segment 6. Segment 9 has also a pair of subterminal spines which are better developed than in *himalayense*.

Respiratory filaments (Plate LII, fig. 17) — About 1.7 mm long, light grey in colour, 6 on each side, arranged in three sessile pairs. The filaments spread out slightly near their origin and then bend slightly downwards. The surface of the filaments is covered with ridges and minute tubercles arranged as in *himalayense*.

Cocoon — It is dirty brown in colour, of the ordinary wall-pocket type, not very compact but there are no windows or spaces in the web, anterior margin thickened into a moderately strong rim. It is on an average 2.9 mm long and its average greatest width at its anterior end is 1.2 mm. It does not cover the pupa completely as its average length at its dorsal end is 2.4 mm.

Described from 17 males and 21 females all bred out of pupæ (Marianbarie, Bengal Tarai, February 1928).

Types and paratypes in my own collection.

The description of the Javanese species *S. iridescens* De Meijere (1913) to some extent applies to this species also. Unfortunately De Meijere's description of the male and female are incomplete and it is not possible to say definitely depending only on his description, that the species described above is a synonym of *S. iridescens*. Although collections have been made from practically all parts of India yet I have so far found this species only at *Marianbarie* (Bengal Tarai). Moreover, while dealing with the Indian Simuliidæ it has been observed that in some cases though in general coloration of the adults looked more or less alike, particularly the male specimens, the terminalia of male and the female and the structure of their pupæ clearly showed that they belonged to distinct species. For this reason it has been thought advisable to describe the above as a new species till such time that the terminalia and the pupa of *iridescens* are definitely known*.

[* Since sending the above to the press Dr F. W. Edwards, sc. d. (Natural History Museum, London), has very kindly sent me specimens of males of some of the Javanese species, identified by him after studying a large collection of simuliids received by him from Java. About the pupa of *S. iridescens* he writes, in a personal communication sent at the same time, 'Pupal filaments 6 in number, all of moderate length and thickness, cocoon closely woven, without windows', but adds, that a peculiarity of *iridescens* female is that the last 4, instead of 3, abdominal tergites are shining. Among the specimens sent by him is a male of *iridescens* and a comparison of its genitalia with those of *nitidithorax* has shown beyond doubt that the latter is quite a distinct species. Moreover, according to Enderlein (1930) *iridescens* belongs to his genus *Odagmia* in which the female claws have a sub-basal tooth, those in *nitidithorax* being simple.]

At first sight the male of *nitidithorax* seems identical with the type of *quiescens* Brunetti but a comparison of their genital armatures, particularly of the inter-coxal pieces of the two clearly shows that they belong to two different species

SUMMARY

It has been found that the terminalia of the female serve as a very useful character for the identification of the different species

Males, females and pupæ of three new species from the Himalayas have been described

Male and pupa of *S. rufibasis* Brunetti have been described for the first time and a revised description of the female has been given

A new variety of *S. rufibasis* based on the ornamentation of the male and the female has also been described

ACKNOWLEDGMENT

I am grateful to the Director, Zoological Survey of India, for lending me for study the type specimens of Brunetti's species of Indian Simuliidæ

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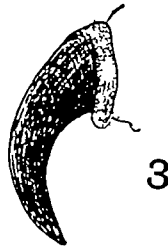
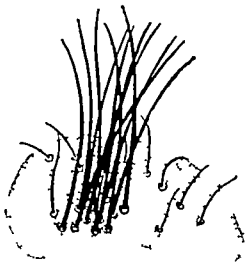
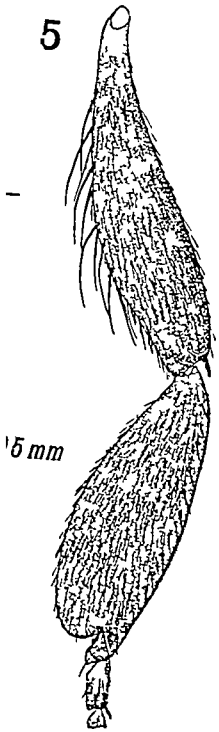
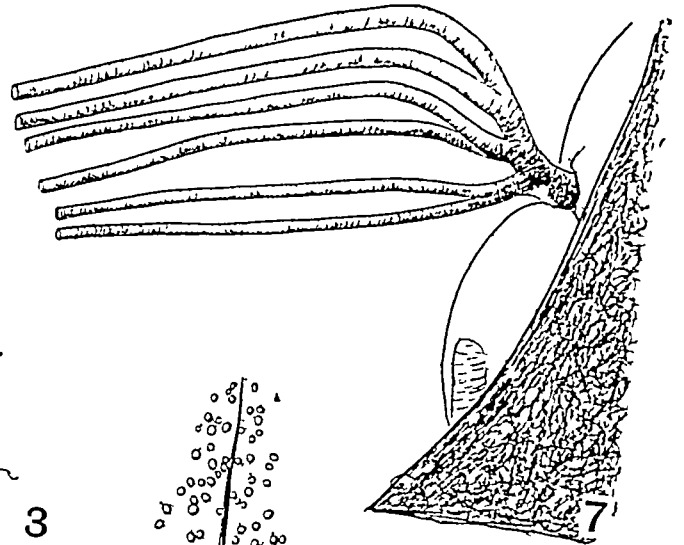
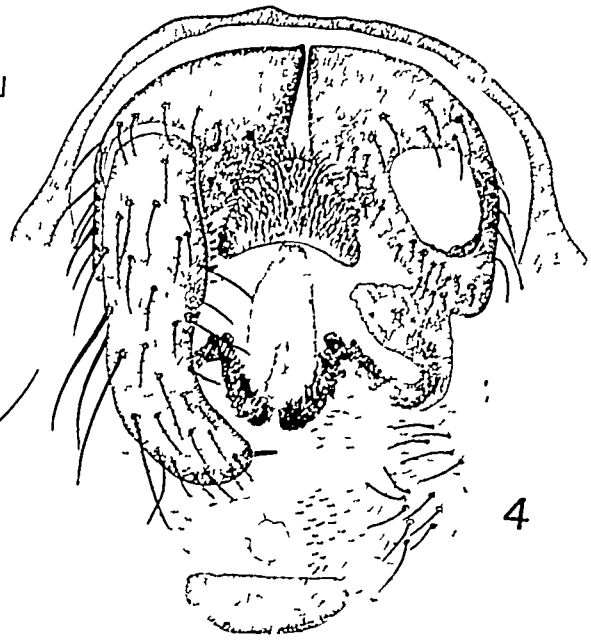
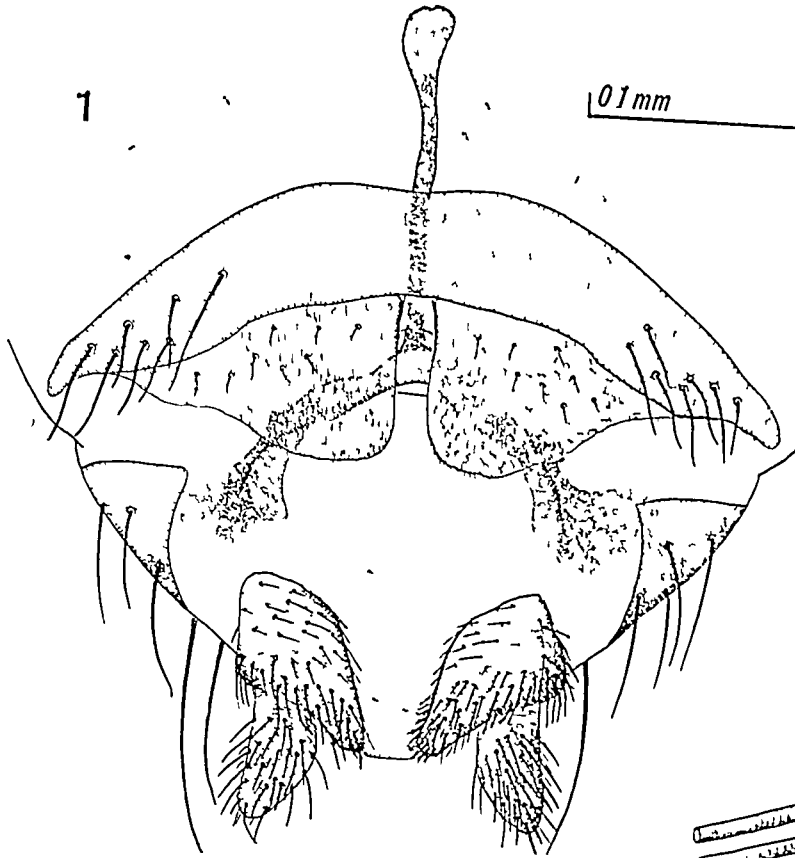
EXPLANATION OF PLATE LI

Simulium (Simulium) rufibasis Brunetti

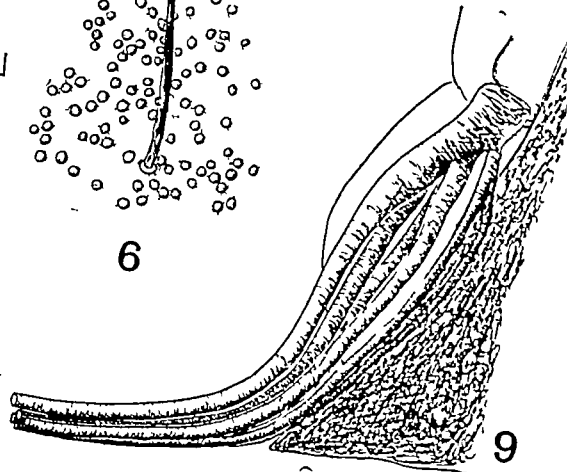
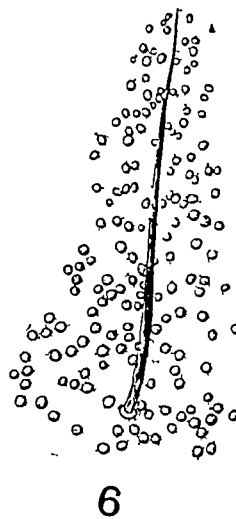
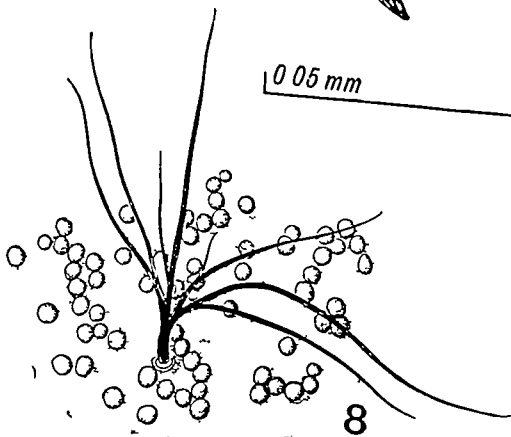
- Fig 1 Ventral view of terminalia of female
 „ 2 Chitinous plate of left side from ventral surface of segment 7, showing the cluster of long black hairs Scale as in Fig 1
 „ 3 A claw from the hind leg of a female
 „ 4 Ventral view of genital armature of a paratype male (left style not shown) Scale as in Fig 1
 „ 5 Tibia, basitarsus and 2nd tarsal segment of right hind leg of a paratype male
 „ 6 One of the dorsal thoracic *trichomes* of pupa , also showing the disc-like tubercles on the integument Scale as in Fig 1
 „ 7 Part of the pupal respiratory filaments of left side in situ Anterior end of pupa and cocoon drawn to show mode of origin of filaments Scale as in Fig 5

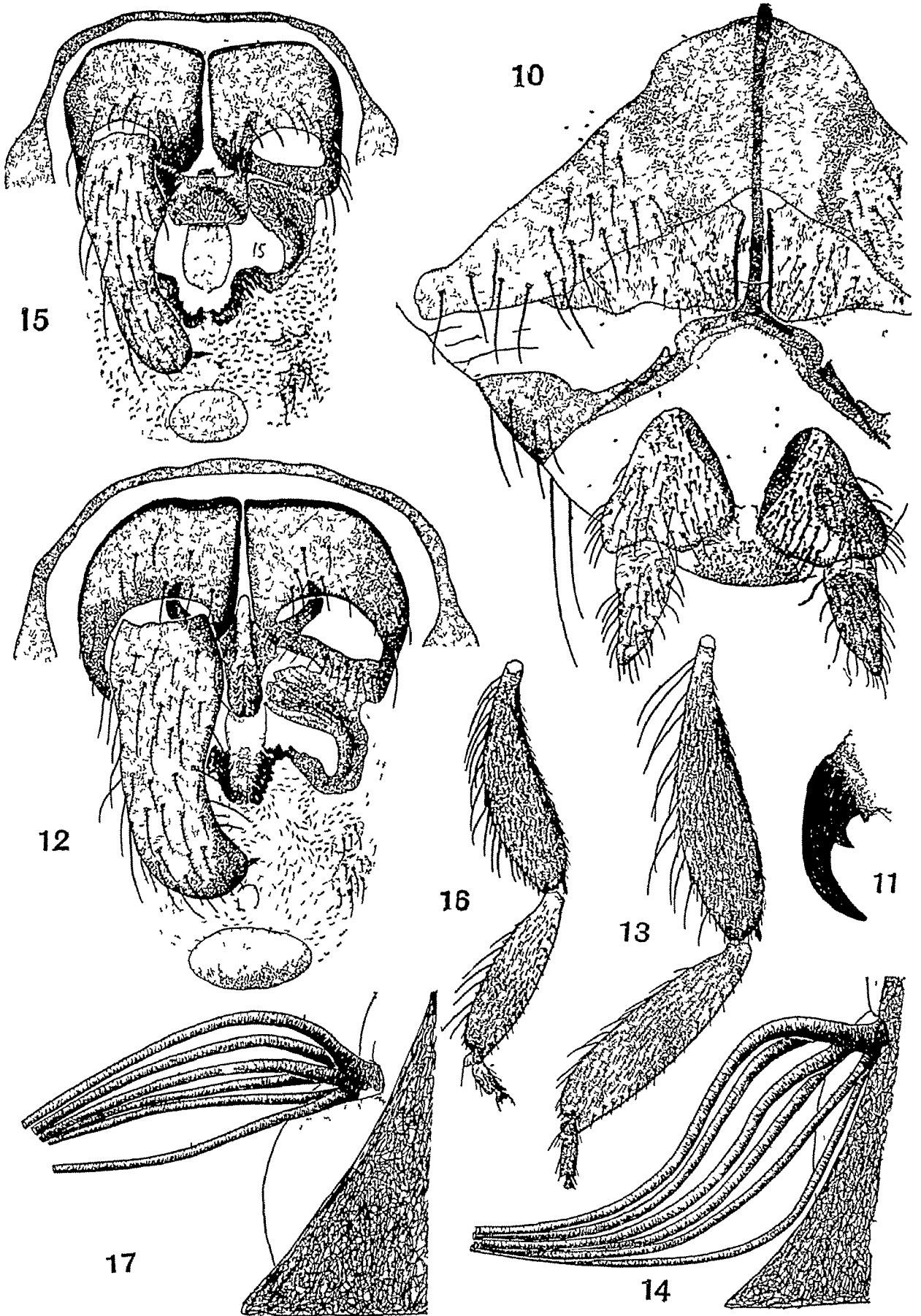
Simulium (Simulium) ramosum sp. n.

- Fig 8 One of the dorsal thoracic *trichomes* of pupa , also showing the disc-like tubercles on the integument Scale as in Fig 1
 „ 9 Part of the pupal respiratory filaments of left side in situ Anterior end of pupa and cocoon drawn to show mode of origin of filaments Scale as in Fig 5



005 mm





EXPLANATION OF PLATE LII

Simulium (Simulium) christophersi sp. n.

- Fig 10 Part of ventral view of terminalia of a paratype female Scale as in Fig 1
 „ 11 A claw from a hind leg of a paratype female Scale as in Fig 3
 „ 12 Ventral view of genital armature of a paratype male (left *style* not drawn)
 Scale as in Fig 1
 „ 13 Tibia, basitarsus and 2nd tarsal segment of a paratype male Scale as in
 Fig 5
 „ 14 Part of pupal respiratory filaments of left side in situ Anterior end of pupa
 and cocoon drawn to show mode of origin of filaments Scale as in Fig 5

Simulium (Simulium) nitidithorax sp. n.

- Fig 15 Ventral view of genital armature of a paratype male (left *style* not
 drawn) Scale as in Fig 1
 „ 16 Tibia, basitarsus and 2nd tarsal segment of right hind leg of a paratype
 male Scale as in Fig 5
 „ 17 Part of pupal respiratory filaments of left side in situ Anterior end of
 pupa and cocoon drawn to show mode of origin of filaments Scale as in
 Fig 5

ANOPHELES BREEDING IN RELATION TO SEASON

BY

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[Received for publication, September 11, 1931]

IN a rural area comprising eight large sized villages near Sonarpur (24-Perganas District, Bengal), a study of the anopheline fauna of all natural collections of water is carried out at regular intervals. All ponds, ditches and drains in the area under observation are serially numbered and each of them is examined systematically twice every month for *Anopheles* larvæ. Larvæ collected from these breeding places are identified in the laboratory and a record is maintained of the results of these fortnightly observations. This work was started in March 1926 and we now have the *Anopheles* breeding history of every pond, ditch and drain in the area for a period of over five years.

An analysis of these records shows that the breeding intensity of the local species of *Anopheles* varies considerably at different seasons of the year and that the different species exhibit a definite seasonal periodicity in breeding. That these variations in the intensity of breeding at different seasons of the year are not mere chance fluctuations is shown by the fact that the breeding curves of the different species repeat themselves year after year. It would be of interest to record these seasonal variations in the breeding intensity of the species of *Anopheles* occurring in this area. This work is based on the results of examination of a very large number of specimens, which total 440,000 during the period 1926-1930.

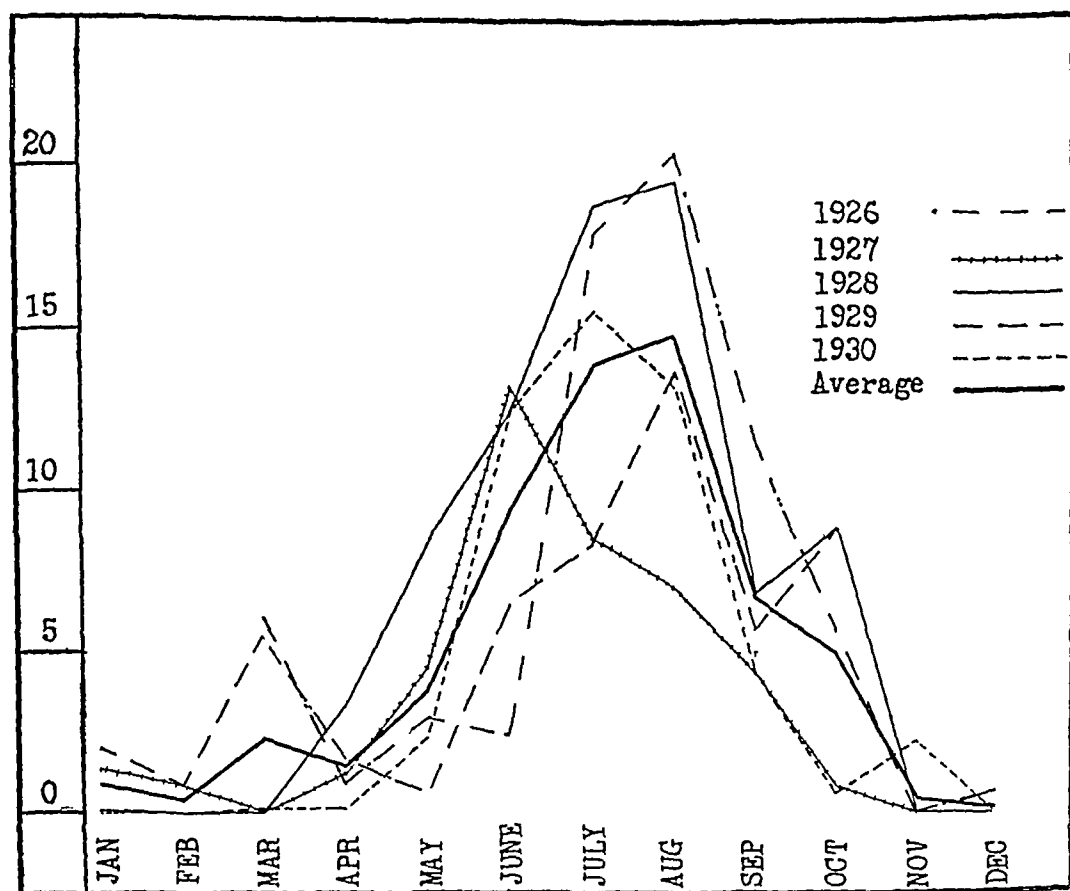
The following eleven species of *Anopheles* occur in the Sonarpur area, namely, *A. subpictus* Grassi, *A. vagus* Don, *A. hyrcanus* var *nigerrimus* Giles, *A. barbirostris* Wulp, *A. pseudogamesi* Strickland and Chowdhury, *A. aconitus* Don, *A. varuna* Iyengar, *A. fuliginosus* Giles, *A. philippinensis* Ludlow, *A. culicifacies* Giles and *A. tessellatus* Theob. Although most of these species have been observed to breed all the year round, each of them has a particular breeding season when its breeding incidence is highest, while during the off season, its incidence is low. In the case of *A. culicifacies* and *A. tessellatus*, the breeding is entirely stopped during their respective off seasons. It will be seen that the high breeding season of one species is often the off season of another.

The total larva catch of each month and the percentage of the number of larvæ of each species to the total for that month form the material for this paper. The monthly records of the number of larvæ of each of the species of *Anopheles* and their percentages for the five years 1926 to 1930 are given in five appendices at the end of the paper, Appendix II to VI. Appendix VII is a total summary of the records of the five years together.

DISTRIBUTION OF RAINFALL

A knowledge of the monthly rainfall at Sonarpur is useful in understanding the variations in the breeding incidence of the several species of the *Anopheles*

CHART 1



Distribution of Rainfall

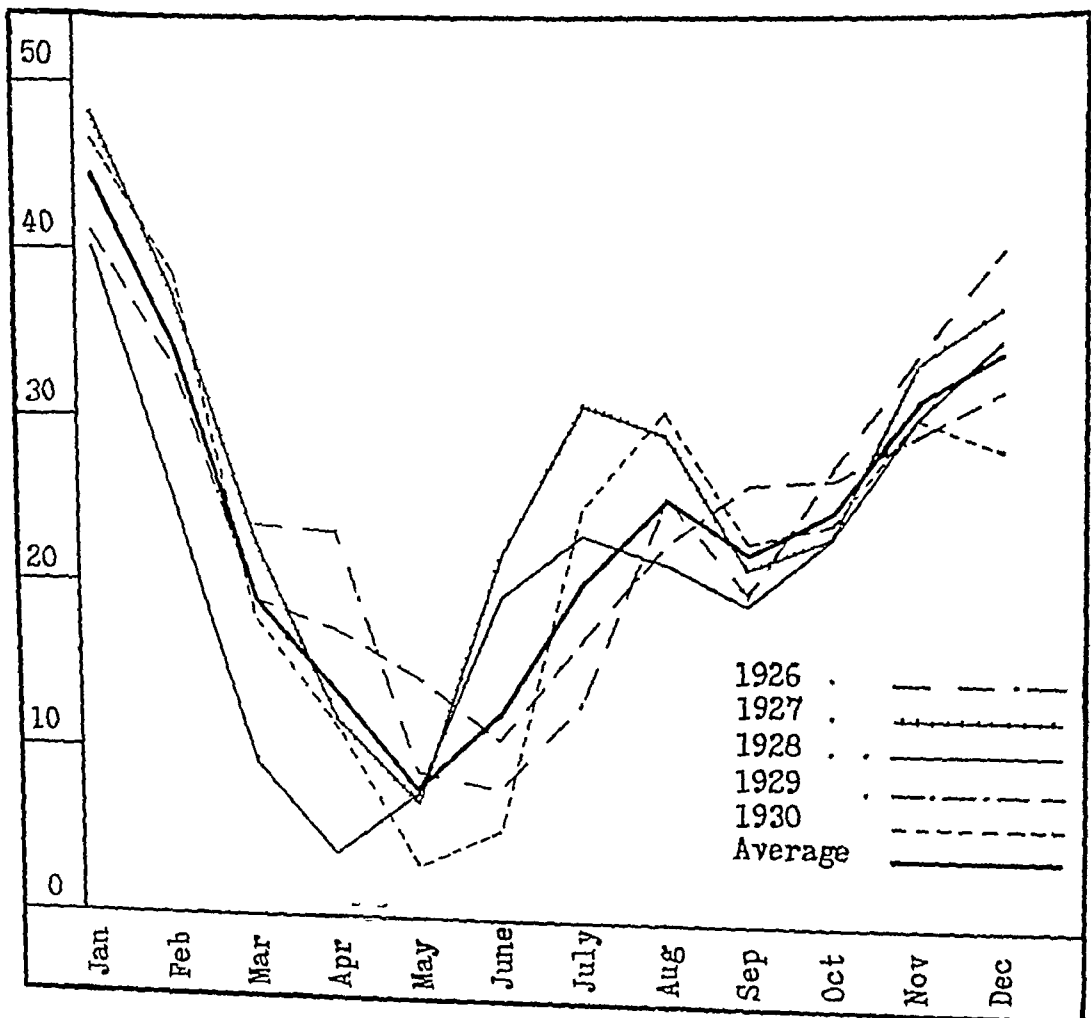
The monthly rainfall at Sonarpur during the five years 1926 to 1930 is represented graphically on Chart 1. The average monthly rainfall is also shown on the same chart. The rainy season starts in June and the three months following, namely July to September, are the wettest months in the year. The rainfall diminishes

in October and during the months November to February, there is practically no rainfall. The three months March, April and May have very little rainfall which is usually less than 2 inches per month on the average. The monthly rainfall record for the five years is given in Appendix I.

THE BREEDING SEASONS

Anopheles hyrcanus var *negerrimus* breeds most heavily during the period November to February, when it forms 30 to 40 per cent of the total larval

CHART 2

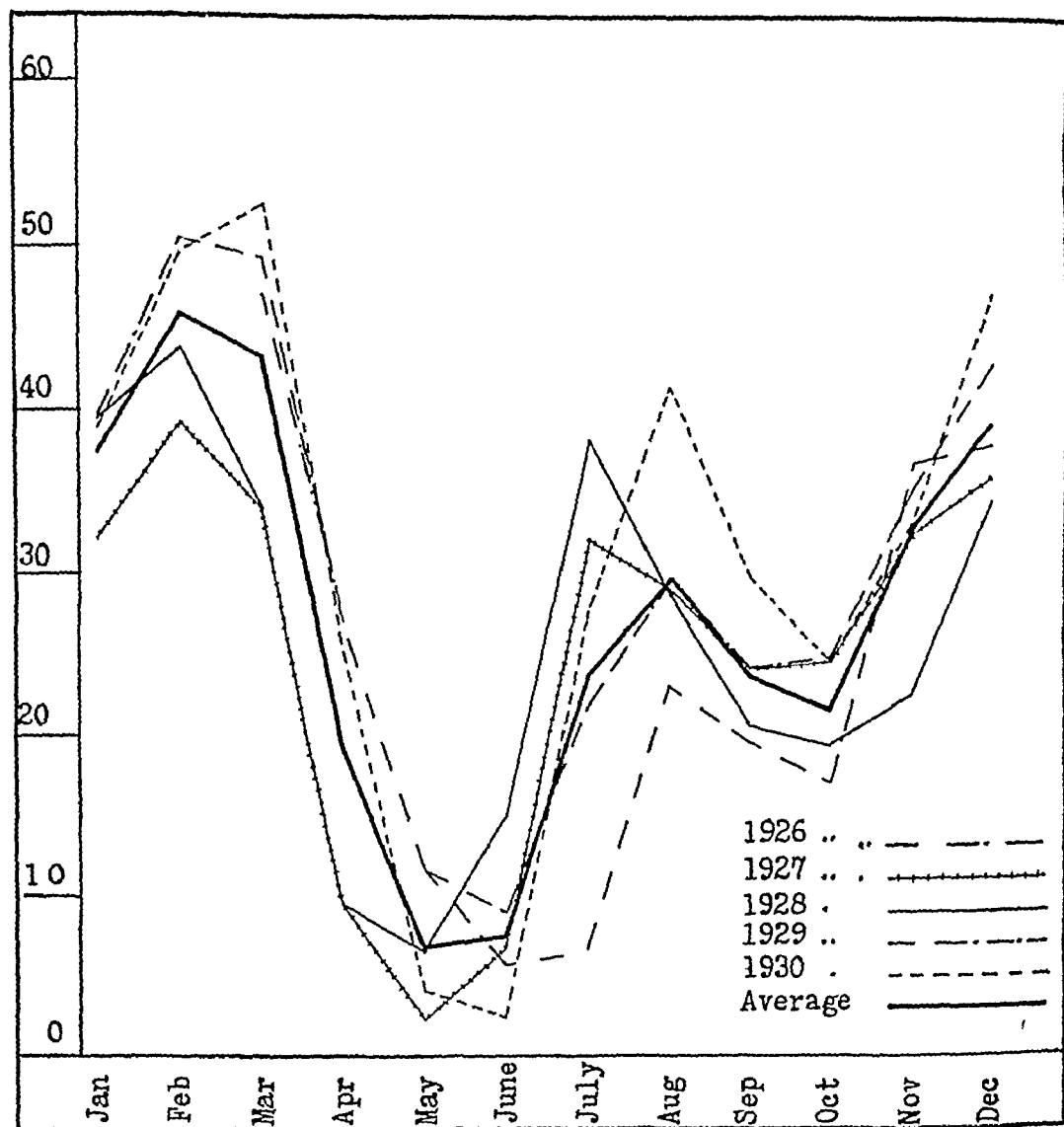


Anopheles hyrcanus

collections (Chart 2). Its breeding incidence falls rapidly in March and reaches its lowest level during April to June. The curve then rises and has a small peak in July and August, but it declines in September. After this month the curve

risers steadily to reach its climax in December and January. These rises and falls in the intensity of breeding of *A. hyrcanus* have been observed during each of the five years 1926 to 1930.

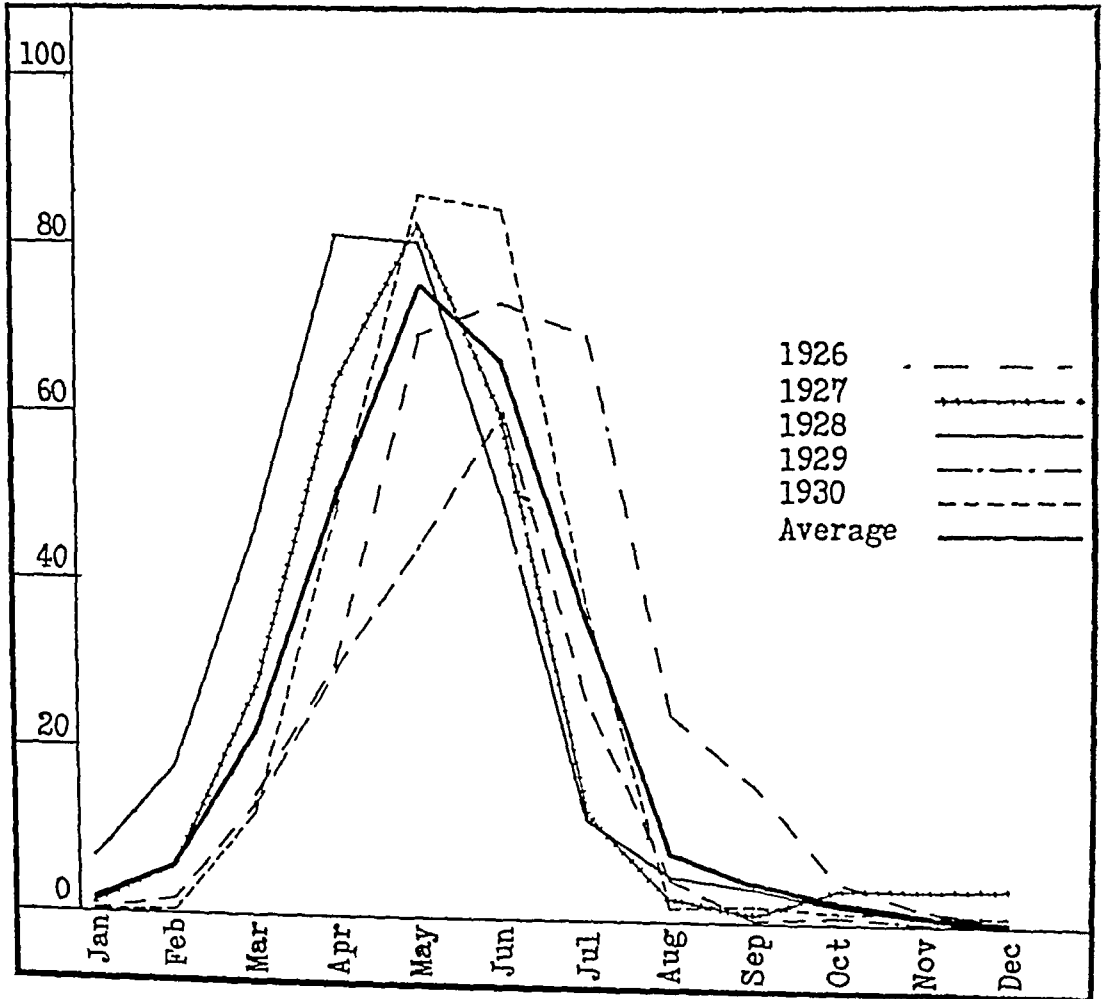
CHART 3

*Anopheles barbirostris*

Anopheles barbirostris breeds in nearly the same types of breeding places as those of *A. hyrcanus* and in many cases the two species occur together. The breeding curve of *A. barbirostris* is very similar to that of *A. hyrcanus* but the two

are not identical. *A. barbirostris* has its heaviest breeding season during February and March, two months later than the high breeding season of *A. hyrcanus*. Its breeding incidence suffers a sudden decline in April (Chart 3) and it is lowest in May and June. After the onset of the rains, the curve rises to a small peak in August,

CHART 4

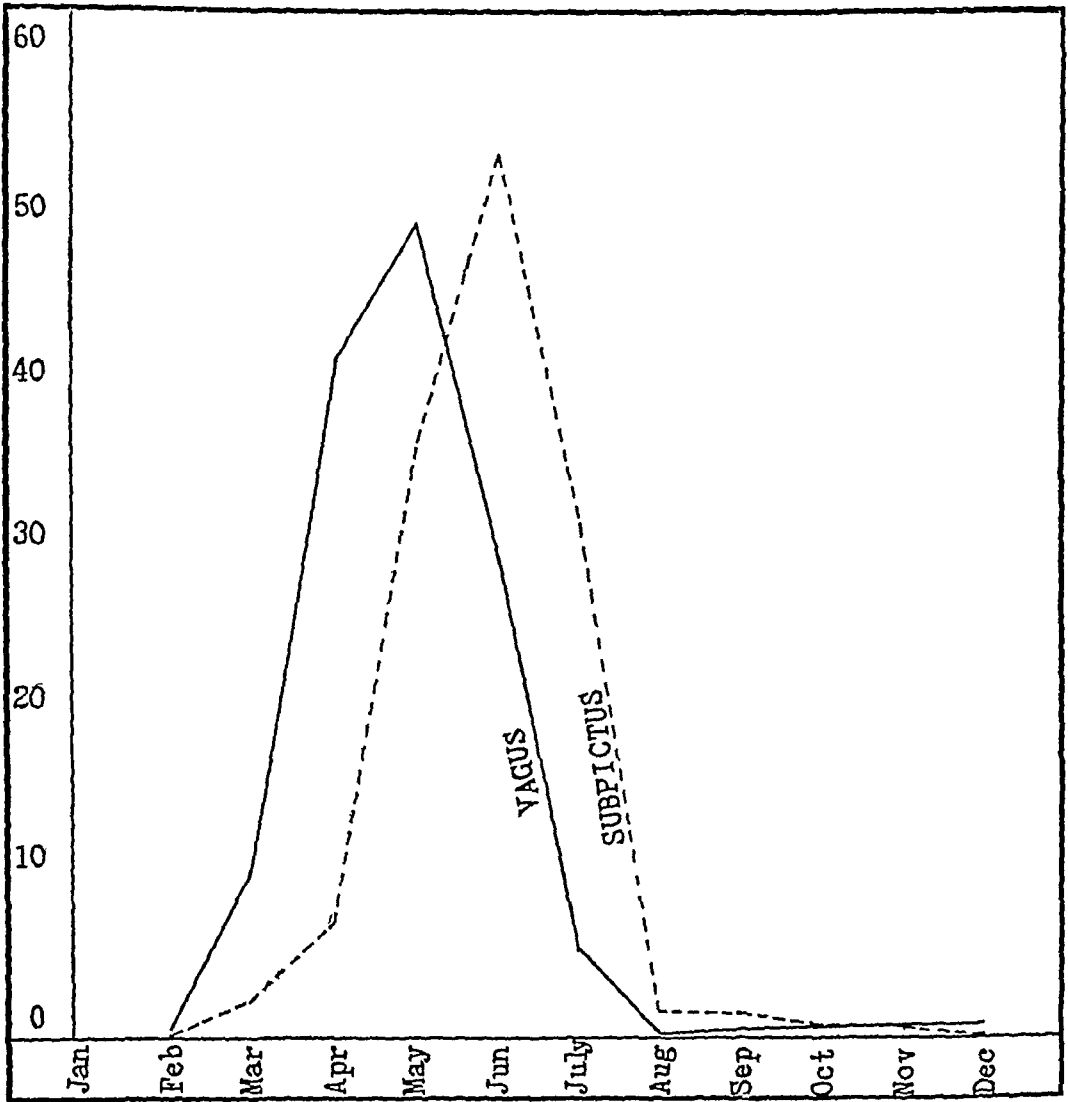
*Anopheles subpictus* and *A. vagus*, 1926-1930

but declines in October, from November the curve rises steadily to reach the climax in the month of February following.

Anopheles subpictus and *A. vagus* the records of these two species for the years 1926 to 1929 were maintained under one head and since the beginning of 1930 they are kept separately. The monthly breeding incidence of the two species together during 1926 to 1930 is represented graphically on Chart 4. They breed

heaviest during the hot season April to June during which period they may constitute more than 80 per cent of the total larva catch After the onset of the rains, their breeding incidence drops suddenly and it reaches a very low figure in August

CHART 5

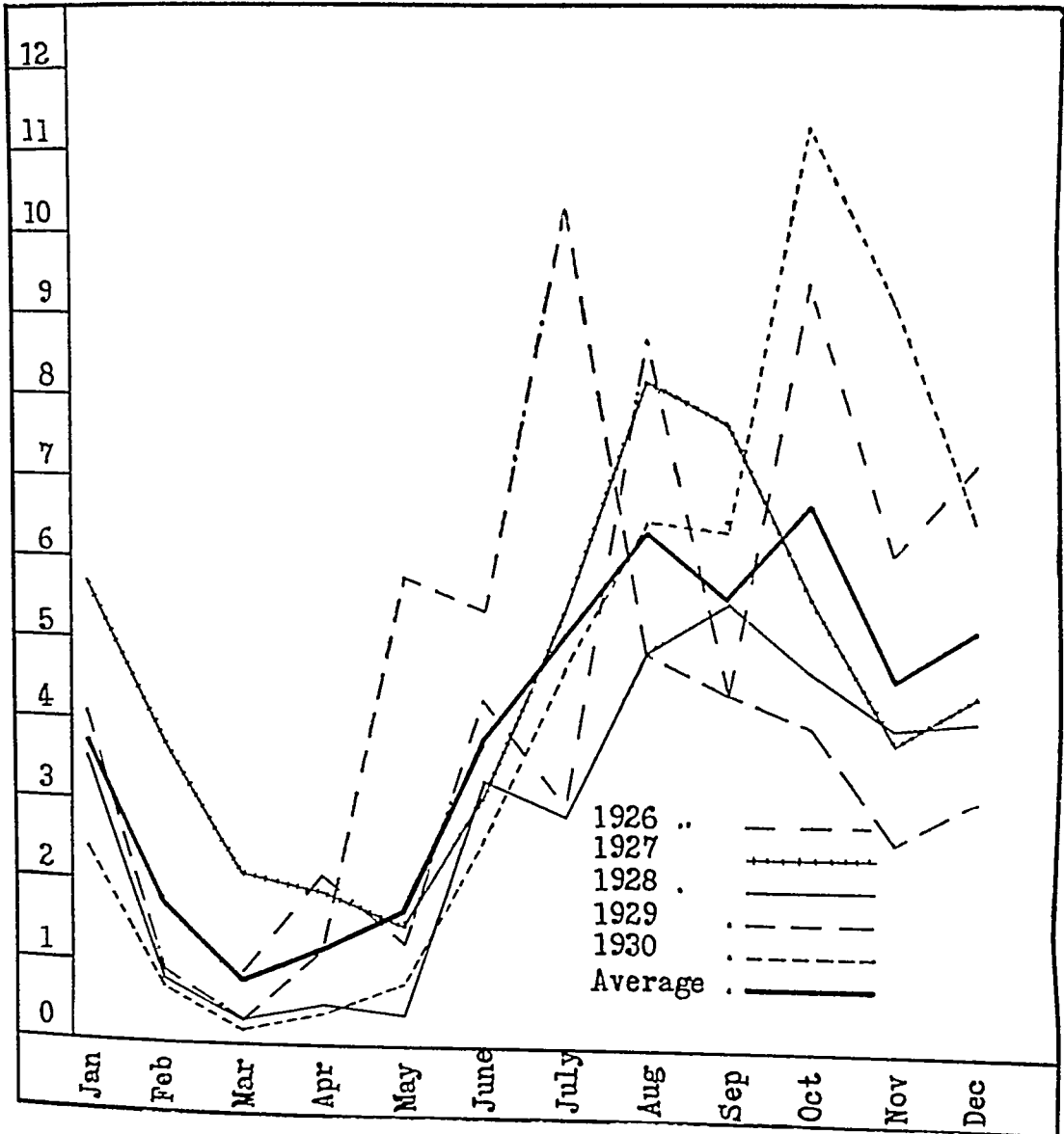


Anopheles subpictus and *A. vagus*, 1930

The fall in the intensity of breeding at this time from a 80 per cent level in May and June to 2 per cent in August is very striking and sudden Between August and January they have a very low breeding incidence being less than 2 per cent

throughout this period. They commence to breed vigorously in March and maintain a very high level during the three months following.

CHART 6

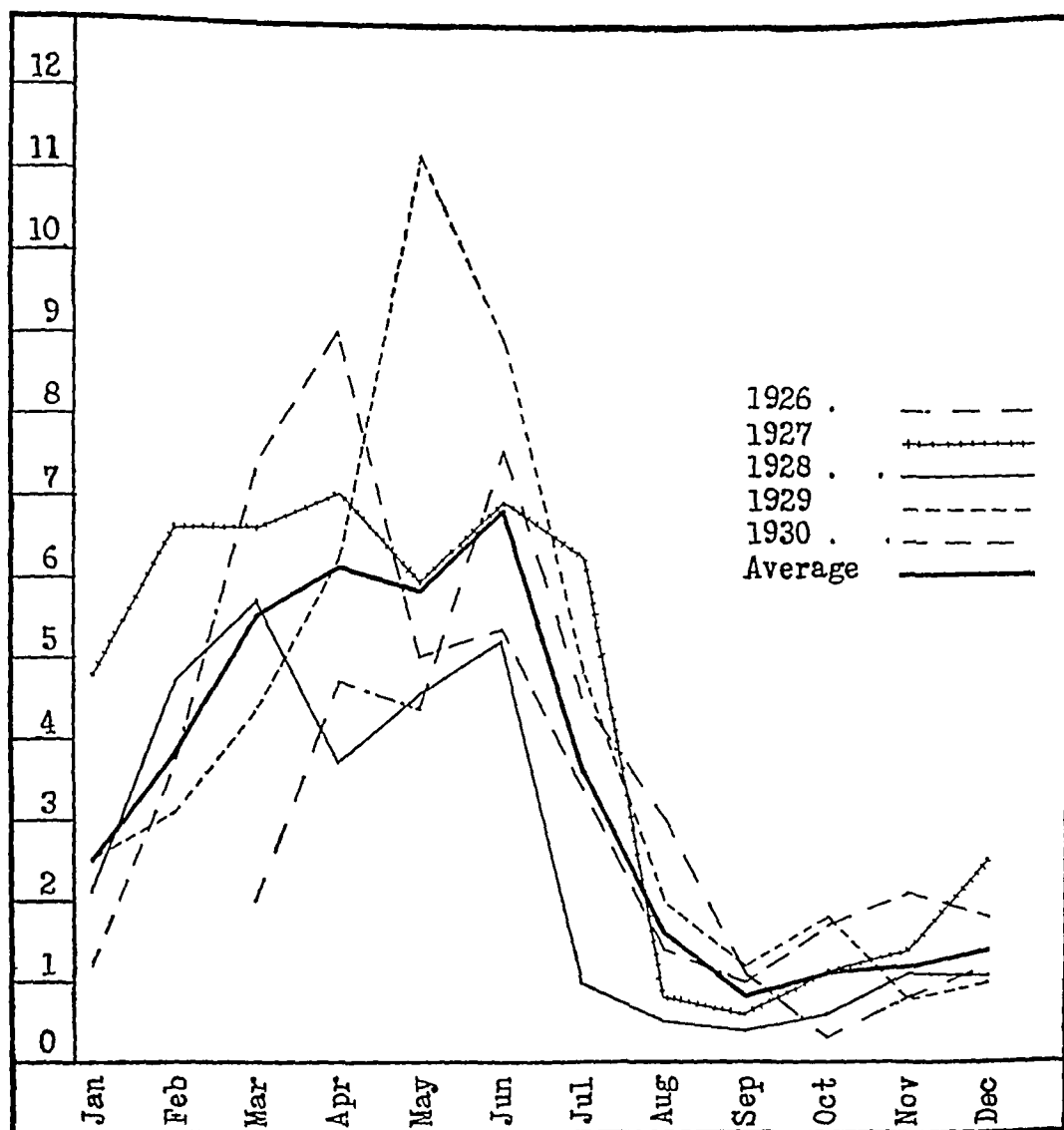


Anopheles pseudogames

Separate records are available for the two species *A. subpictus* and *A. vagus* for the year 1930. Chart 5 represents the breeding incidence of the two species during 1930 in separate curves. It will be observed that although both of them

have a heavy breeding season during summer, they exhibit some differences *Anopheles vagus* starts breeding earlier than *A. subpictus* which follows it a month later. The maximum breeding incidence of *A. vagus* is during the early summer

CHART 7



Anopheles fuliginosus and *A. philippinensis*

months, April to June, during which period there is not much rainfall. It declines with the onset of the early rains. The breeding season of *A. subpictus* starts a month later and reaches its height during May to July. Its breeding season

corresponds closely with the early rains that occur in May and June, it declines markedly as soon as heavy rains commence in July

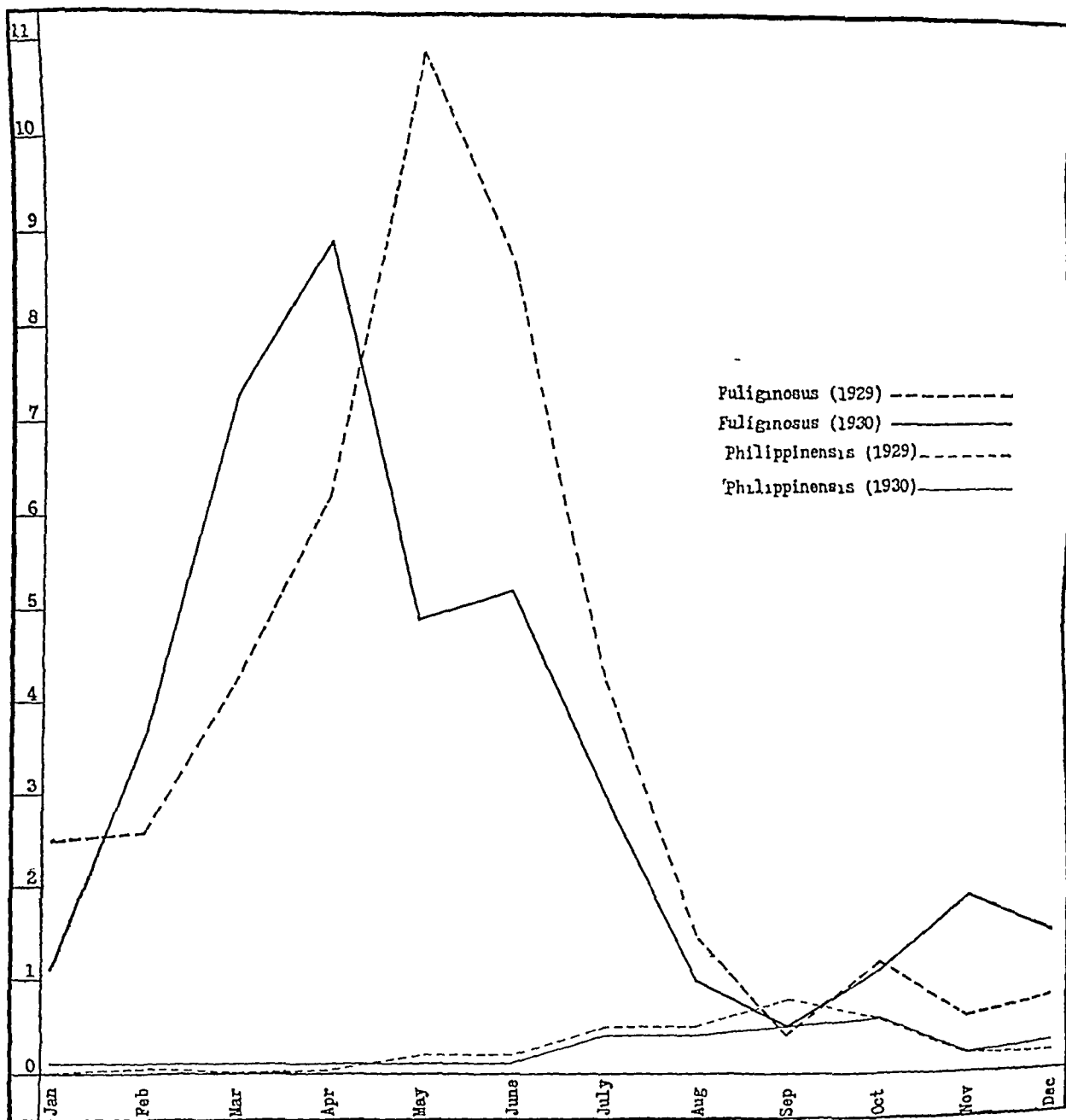
Anopheles pseudojamesi, although fairly common, has not been observed to breed in large numbers at any time. Its highest incidence during its breeding season is about 10 per cent of the total catch, while at other times it is usually less than 4 per cent. Its breeding season starts about the middle of the rainy season and during August to October, it forms from 6 to 10 per cent of the total catch. After October, its breeding intensity diminishes and it reaches a very low level during February to May when it is less than 2 per cent of the total.

Anopheles fuliginosus and *A. philippinensis* for part of the period of observation, records of these two species were maintained together under one head, while during the later years, they were recorded separately. The seasonal incidence of the two species taken together is represented graphically on Chart 7. These species do not occur in large numbers at any time and even during their breeding season, they do not rise above the 10 per cent level. The combined figures for the two species show an increased incidence in breeding during the months March to June, during August to December, the curve is low, being less than 2 per cent throughout that period. The same trend of the breeding curve is observed during every one of the years 1926 to 1930.

When the two species are considered separately, their respective intensity of prevalence as also their individual breeding seasons are brought out. Separate records for the two species are available for the two years 1929 and 1930 and Chart 8 is based on these records. In regard to numerical prevalence, *A. fuliginosus* is the more common of the two species and *A. philippinensis* occurs only in sparse numbers in this area. One difference in the breeding curves of the two species is striking while *A. fuliginosus* breeds most during March to June and subsequently declines to reach its lowest level in September, *A. philippinensis* has a very low incidence during the dry season and breeds in greater numbers during the wet months July to October, with its highest breeding during September. The two species although very closely allied have different breeding seasons at Sonarpur.

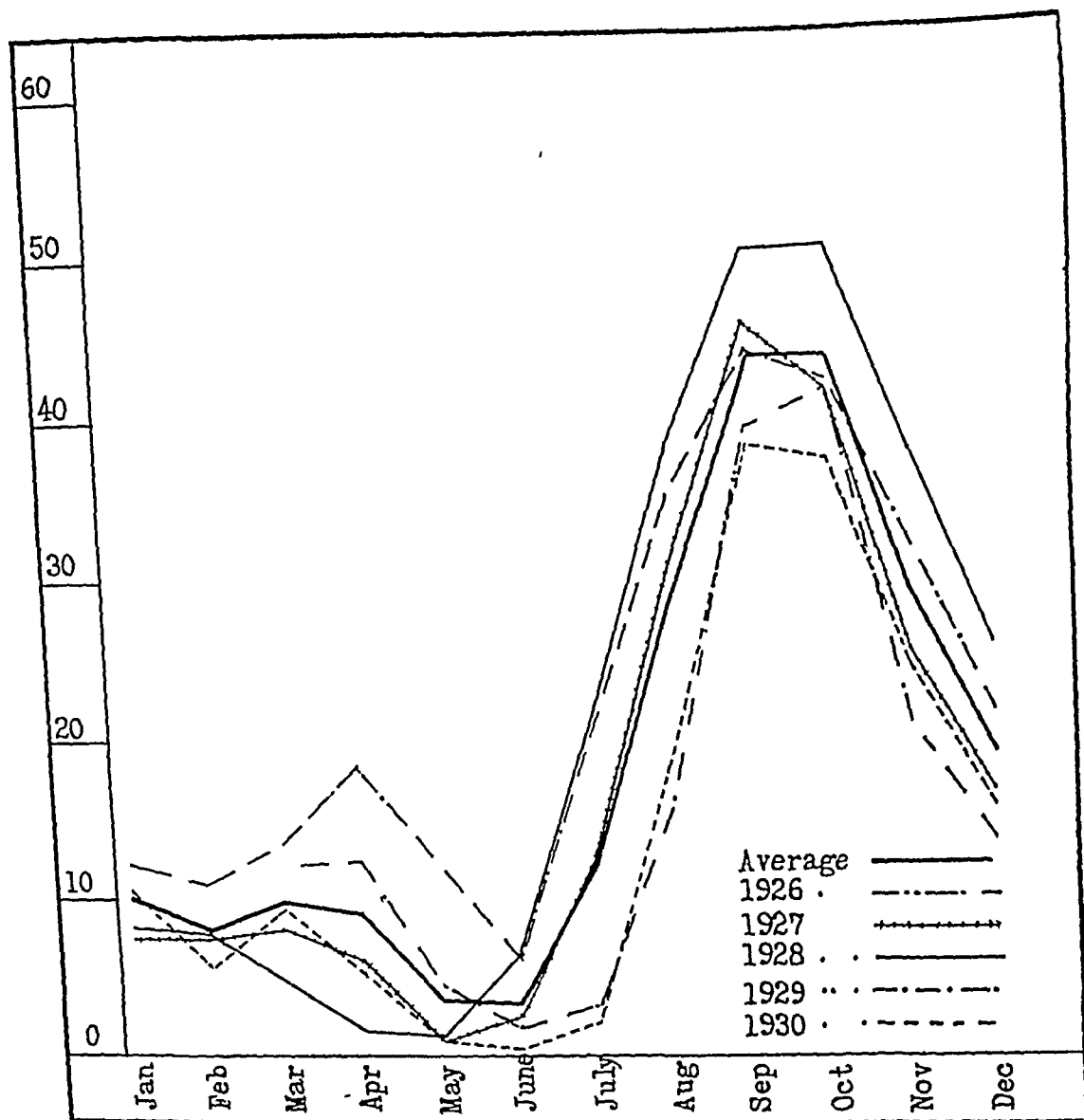
Anopheles varuna and *A. aconitus* records of these two species were maintained together during the period 1926 to 1929. In 1930, they were recorded separately. The combined records of the two species for the five years show that these species have an intense breeding season during August to November and their lowest incidence is during May and June. Their breeding intensity rises abruptly in July after the onset of the rains and between August and October, they constitute nearly half of the total larva catch. The breeding incidence commences to decline

CHART 8

*Anopheles fuliginosus* and *A. philippinensis*, 1929-1930

in November and is low during the months January to June May and June are the months when it is at the lowest level

CHART 9

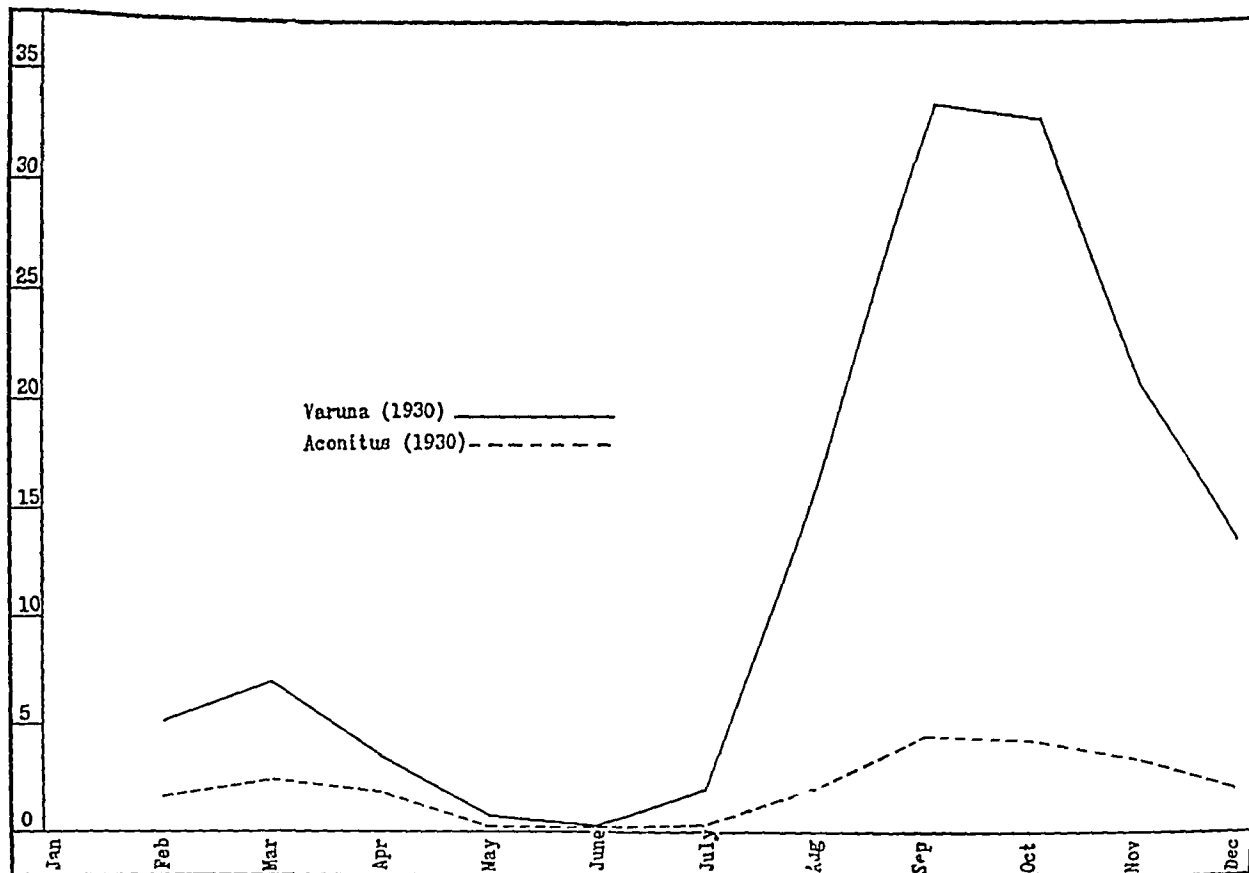


Anopheles varuna and *A. aconitus* (contd)

Separate figures of the two species *A. aconitus* and *A. varuna* are available for the year 1930. The variations in the breeding intensity of these two species are separately represented on Chart 10. Of the two species, *A. varuna* is decidedly the more common one while *A. aconitus* has only a low incidence. They, however,

have a close similarity as regards breeding periodicity. The curves of both species are lowest in May and June and they rise together after the onset of the rains and reach their maximum in September. Both the curves decline after September and reach a low level during the dry months.

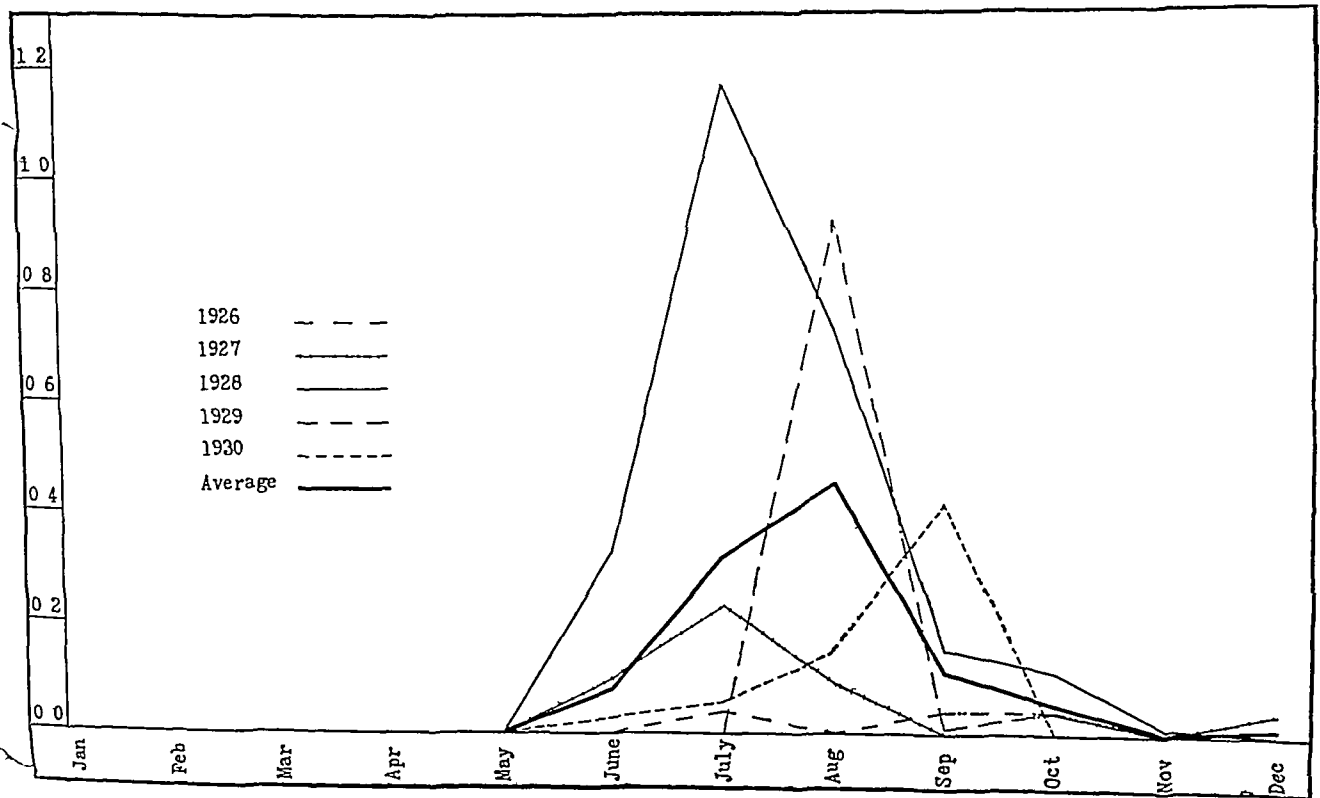
CHART 10

*Anopheles varuna* and *A. aconitus*, 1930

Anopheles tessellatus is a rare species in the area as compared with other species of *Anopheles*. Even during the height of the season, its incidence is not more than one per cent. The monthly breeding incidence of *A. tessellatus* during 1926 to 1930 is represented on Chart 11. This species has a restricted breeding season and even then it occurs only in small numbers, during the off season it has not been observed.

to breed at all. It starts breeding in June, reaches its peak between July and September, after which its incidence declines. After October, no larvæ of this species have been found in this area till the following June. While the other species of *Anopheles* that occur in Sonampur have been observed to breed all the

CHART 11.

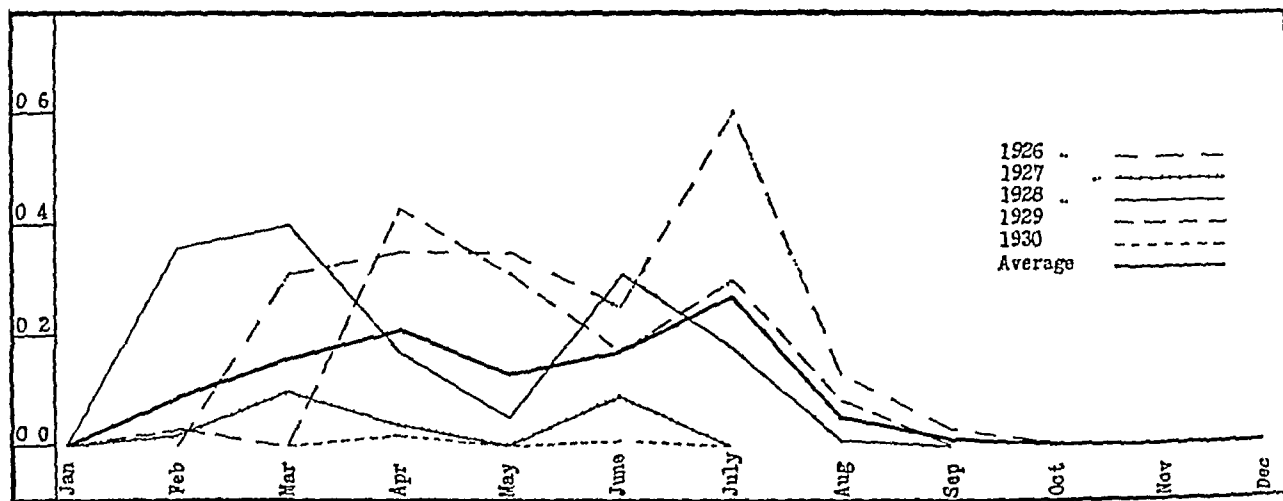


Anopheles tessellatus

year round, although only in small numbers during their respective off seasons, *A. tessellatus* has not been observed to breed at all during its off season. In this respect it behaves like *A. culicifacies* which also stops breeding during its off season.

Anopheles culicifacies the incidence of this mosquito in the area is still lower than that of *A. tessellatus*. It has been observed as larvæ mostly between March and July and it has not been observed to breed during October to January. The breeding incidence of this species during the years 1926 to 1930 is represented on Chart 12. These curves are based on small numbers of larvæ as this species has not been found in large numbers at any time. Even when its breeding is highest, its incidence is very much below 1 per cent of the total larva catch.

CHART 12.



Anopheles culicifacies

SUMMARY

The studies on the seasonal breeding incidence of the eleven species of *Anopheles* in the Sonarpur area show that each of them has a definite breeding periodicity, a breeding season when its incidence is high and an off season when its incidence is low. These seasonal fluctuations have been observed during each of the five years this study has been in progress. The results are briefly summarized below.

Anopheles vagus breeds heaviest during the hot season, April to June, the breeding season of *A. subpictus* is during May to July. The former is a dry season breeder and the latter is an early rains breeder. *A. hyrcanus* is an early winter breeder, with its highest incidence during December and January.

A. barbirostris is a late winter breeder with its high incidence in February and March. *A. pseudojamesi* breeds most during the wet season and its incidence is highest during August to October. *A. fuliginosus* is a dry season breeder with its peak of prevalence in April to May. *A. philippinensis* occurs in small numbers, its breeding season is during the wet months and it breeds most in September. *A. tessellatus* is a species which is sparse here. It breeds during the wet season and entirely stops breeding from November to May. *A. culicifacies* is a dry season breeder and occurs mostly during March to July. During the other months of the year, it is practically absent. *A. varuna* and *A. aconitus* are wet season breeders and breed most during August to November. The latter species occurs in very small numbers compared to *A. varuna*.

CONCLUSION

These observations are of more than mere academic interest. They seem to be of practical importance in connection with epidemiological studies. In a locality one frequently finds more than a single species of *Anopheles* which is capable of transmitting malaria. But it is often difficult to determine which of the several carrier species that occur there are the actual transmitters of malaria. Let us now consider the Sonarpur area. Six or even seven species of *Anopheles* out of the 11 species observed in this area are known transmitters of malaria, namely, *A. varuna*, *A. aconitus*, *A. fuliginosus*, *A. philippinensis*, *A. culicifacies*, *A. tessellatus* and *A. hyrcanus*. One cannot say that all the seven species mentioned above act as actual transmitters of malaria in the area. Nor is it easy to say that any particular species is the most important carrier except it be after a very large series of dissections of all these species. In this respect, a knowledge of the seasonal incidence of the different species of *Anopheles* is of value. The relation of the period of prevalence of the carrier species to the malaria season would bring out their importance as an actual transmitter of malaria in the locality. A carrier species which breeds in close conjunction with the malaria season is one which in all probability is an actual transmitter whereas one which breeds during the non-malarial season is, in all probability, of very little importance as an actual transmitter in the particular locality, even though it may be a species known to be capable of transmitting the parasite.

In this perspective, let us consider the seven carrier species of *Anopheles* and their breeding seasons in relation to the malaria season which is September to November. *Anopheles fuliginosus* is a known transmitter, but its incidence in the Sonarpur area does not correspond with the malaria season and as such, although it is capable of transmission, it does not appear to be a likely transmitter. *Anopheles philippinensis* on the other hand breeds during the wet season immediately preceding the malaria season and this species is, in all probability, an

actual transmitter in this area *A. culicifacies*, although an efficient transmitter, does not seem to be of any importance owing to the fact that the season of high incidence of this species is very different from the malaria season. On the other hand, the close relation between the malaria season and the breeding incidence of *A. tessellatus* seems to indicate that even though this species occurs only in small numbers, it may yet transmit malaria. *A. hyrcanus* is known to be capable of transmitting malaria. This species has a winter rise after the close of the malaria season, at a time when the incidence of malaria is low. Judged from this fact, this species is probably not concerned in malaria transmission at Sonarpur.

Two more species to be discussed are *A. varuna* and *A. aconitus*, both of which have their highest incidence between August and November. Their breeding seasons correspond closely with the malaria season and it seems very likely that these two species play a considerable part in the transmission of malaria in this locality.

The present studies are thus helpful in understanding the likely transmitters among the several carrier species that may occur in an area. Out of the seven carrier species observed at Sonarpur, the rise in the incidence of the species corresponds to the malarial season in the case of four species, namely, *A. aconitus*, *A. varuna*, *A. philippinensis* and *A. tessellatus* whereas in the case of *A. culicifacies*, *A. fuliginosus* and *A. hyrcanus*, the rise in their incidence does not correspond to the malaria season. This indicates that the former four species are likely to be actual carriers in the area, whereas the latter three carrier species which have a very low incidence during the malaria season and the period preceding it, and a high incidence during the non-malarial season are evidently not likely to be carriers of importance in the area.

To judge the relative importance of the four species here considered to be the likely transmitters, two factors need to be considered. The first factor is the relative incidence of these species during their season of maximum prevalence. The curve of *A. varuna* rises above the 30 per cent level and this species is undoubtedly the most prevalent of the four. Next to it comes *A. aconitus* in which the maximum prevalence is about 4 per cent. Lastly come *A. philippinensis* and *A. tessellatus*, in both of which the maximum incidence is one per cent and under. The second factor to be considered is the degree of susceptibility of these carrier species to infection with malaria parasites. From a consideration of these two factors, namely, the numerical prevalence and the susceptibility to infection, of those carrier anophelines which show a relatively high prevalence during the malaria season, it should be possible to appraise the relative importance of carrier species in any individual area.

APPENDIX I

Monthly rainfall record at Sonarpur, 1926-30

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
1926			61	09	29	24	178	203	113	57	00	07
1927	13	08	00	12	45	131	85	70	43	08	00	00
1928	01	00	00	33	84	124	187	194	68	88	00	00
1929	20	08	55	17	07	64	83	136	57	88	00	00
1930	00	00	01	01	23	124	155	132	43	06	22	00
AVERAGE	085	04	23	14	38	93	138	117	67	49	04	01

APPENDIX II
Anopheles larvæ collected during 1926
 Monthly figures and percentages

	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
<i>subpictus</i> and <i>vagus</i>	1 050 14 1%	1,926 30 4%	5,230 68 9%	5,856 72 7%	5,157 69 0%	2,088 24 3%	1,270 16 3%	246 4 8%	156 1 8%	27 0 3%
<i>hyrcanus</i>	1,748 23 5%	1,526 23 3%	672 8 8%	631 7 8%	976 13 1%	2,147 25 2%	1,512 19 5%	1,390 27 0%	2,941 33 8%	3,233 39 9%
<i>barbirostris</i>	3,489 47 0%	1,759 26 8%	906 11 9%	460 5 7%	480 6 4%	1,939 22 9%	1,521 19 6%	883 17 1%	3,199 36 8%	3,064 37 8%
<i>pseudoparvus</i>	67 0 9%	138 2 1%	103 1 3%	318 4 3%	227 3 0%	714 8 7%	313 4 4%	482 9 4%	530 6 1%	585 7 2%
<i>varuna</i> and <i>aconitus</i>	903 12 2%	811 12 4%	343 4 5%	140 1 7%	249 3 3%	1 334 15 6%	3,035 39 1%	2,134 41 4%	1,806 20 8%	1,097 13 5%
<i>fuliginosus</i> and <i>philippinensis</i>	146 2 0%	306 4 7%	335 4 4%	602 7 5%	340 4 5%	253 3 0%	85 1 1%	14 0 3%	68 0 8%	96 1 2%
<i>culicifacies</i>	23 0 31%	23 0 35%	27 0 35%	20 0 25%	45 0 60%	11 0 13%	2 0 03%	0 0 04%	0 0 04%	0 0 04%
<i>tesellatus</i>	0	0	0	0	3 0 04%	0	3 0 04%	3 0 04%	0	0

Anopheles larvae collected during 1927

Monthly figures and percentages

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
<i>subpictus</i> and <i>vagus</i>	109 1.4%	507 5.8%	2,295 26.7%	4,956 63.7%	6,487 82.3%	3,429 39.0%	273 12.7%	179 2.7%	79 1.0%	182 4.0%	339 4.0%	384 4.3%
<i>hyrcanus</i>	3,594 48.2%	3,263 37.2%	1,946 22.6%	919 11.8%	544 6.9%	1,244 21.4%	661 30.7%	1,922 28.9%	1,793 21.1%	1,055 23.2%	2,822 33.3%	3,265 36.6%
<i>barbrosistris</i>	2,417 32.3%	3,436 39.2%	2,918 33.9%	742 9.5%	174 2.2%	397 6.8%	690 32.1%	1,930 29.0%	2,052 24.1%	1,115 24.6%	2,738 32.3%	3,192 35.9%
<i>pseudojamesi</i>	427 5.7%	324 3.7%	178 2.1%	147 1.9%	117 1.5%	182 3.1%	116 5.4%	543 8.2%	655 7.7%	255 5.6%	322 3.8%	390 4.4%
<i>varuna</i> and <i>aconitus</i>	535 7.4%	655 7.5%	689 8.0%	470 6.0%	97 1.2%	148 2.5%	272 12.6%	2,021 30.4%	3,879 45.6%	1,885 41.5%	2,138 25.2%	1,471 16.5%
<i>fuliginosus</i> and <i>philippi- nensis</i>	360 4.8%	575 6.6%	568 6.6%	544 7.0%	463 5.9%	402 6.9%	134 6.2%	53 0.8%	48 0.6%	48 1.1%	122 1.4%	223 2.5%
<i>culicifacies</i>	0	2 0.02%	9 0.10%	3 0.04%	0	5 0.09%	0	0	0	0	0	0
<i>tessellatus</i>	0	0	0	0	0	6 0.10%	5 0.23%	6 0.09%	0	0	0	4 0.04%

APPENDIX IV

Anopheles larvæ collected during 1928
Monthly figures and percentages

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
<i>subpictus</i> and <i>vagus</i>	416 6 5%	1,072 17 7%	2,534 45 9%	5,316 80 7%	5,059 79 9%	2,883 50 3%	924 12 1%	421 5 7%	410 4 3%	190 2 0%	86 0 9%	23 0 3%
<i>hyrcanus</i>	2,578 40 2%	1,499 24 8%	514 9 3%	249 3 8%	462 7 3%	1,100 19 2%	1,354 22 9%	1,587 21 1%	1,818 19 1%	2,163 23 0%	2,934 29 9%	3,176 34 8%
<i>barburostris</i>	2,529 39 5%	2,645 43 8%	1,877 34 0%	633 9 5%	109 6 5%	867 15 1%	2,601 38 3%	2,132 28 7%	1,948 20 5%	1,921 19 3%	2,396 22 4%	3,124 31 2%
<i>pseudojanensis</i>	225 3 5%	50 0 8%	14 0 3%	32 0 5%	27 0 4%	189 3 3%	196 2 9%	364 4 9%	518 5 5%	438 4 7%	392 4 0%	378 4 1%
<i>varuna</i> and <i>aconitus</i>	523 8 2%	473 7 8%	252 4 6%	109 1 6%	80 1 3%	364 6 3%	1,432 21 4%	2,828 38 1%	4,760 50 1%	4,711 50 3%	3,701 37 7%	2,323 25 5%
<i>fuliginosus</i> and <i>philip- pinensis</i>	136 2 1%	282 4 7%	313 5 7%	217 3 7%	286 4 5%	297 5 2%	69 1 0%	35 0 5%	36 0 4%	55 0 6%	105 1 1%	102 1 1%
<i>culicifacies</i>	0	22 0 36%	22 0 40%	11 0 17%	3 0 05%	19 0 31%	12 0 18%	1 0 01%	0	0	0	0
<i>tessellatus</i>	0	0	0	0	0	19 0 33%	79 1 16%	54 0 73%	14 0 15%	10 0 11%	1 0 01%	0

APPENDIX V
Anopheles larvæ collected during 1929
Monthly figures and percentages

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept.	Oct	Nov	Dec
<i>subpictus</i> and <i>vagus</i>	25 0.4%	143 2.0%	947 13.5%	2,519 29.8%	2,270 44.3%	1,529 39.8%	1,539 25.8%	636 5.3%	51 0.5%	65 1.0%	17 0.4%	26 0.2%
<i>hyrcanus</i>	2,868 41.1%	2,300 32.9%	1,339 19.0%	1,449 17.2%	730 14.4%	819 10.9%	1,006 16.8%	2,709 22.4%	2,829 26.1%	1,762 26.3%	3,837 29.1%	4,208 31.7%
<i>barbirostris</i>	2,775 39.7%	3,538 50.6%	3,474 49.3%	2,273 26.9%	597 11.7%	680 9.0%	1,284 21.5%	3,577 29.5%	2,611 24.1%	1,657 24.7%	1,651 25.2%	5,656 42.7%
<i>pseudojamesi</i>	285 4.1%	65 0.9%	24 0.3%	99 1.2%	296 5.8%	406 7.4%	615 10.3%	589 4.9%	478 4.4%	268 4.0%	344 2.6%	411 3.1%
<i>varuna</i> and <i>aconitus</i>	855 12.2%	767 11.0%	952 13.5%	1,544 18.3%	629 12.4%	469 6.2%	1,225 20.5%	1,243 35.0%	4,765 43.9%	2,823 42.1%	4,232 32.0%	2,832 21.4%
<i>fuliginosus</i>	177 2.5%	179 2.6%	305 4.3%	522 6.2%	555 10.9%	650 8.6%	277 4.3%	187 1.5%	41 0.4%	80 1.2%	79 0.6%	103 0.8%
<i>philippinensis</i>	0	4 0.05%	0	3 0.03%	12 0.24%	12 0.16%	29 0.49%	54 0.45%	81 0.75%	41 0.61%	18 0.14%	24 0.18%
<i>culicifacies</i>	0	2 0.03%	0	36 0.43%	16 0.31%	13 0.17%	18 0.30%	10 0.08%	0	0	0	0
<i>tessellatus</i>	0	0	0	0	0	0	0	112 0.92%	1 0.01%	3 0.04%	0	0

APPENDIX VI
Anopheles larvae collected during 1930
 Monthly figures and percentages

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
<i>subpictus</i>	0	15 0 2%	157 2 4%	462 7 0%	3,172 36 0%	3,827 53 9%	1,692 31 6%	100 1 7%	126 1 6%	51 0 8%	40 0 7%	11 0 2%
<i>vagus</i>	6 0 1%	26 0 4%	686 10 3%	2,744 41 6%	4,382 49 8%	2,127 30 0%	299 5 6%	11 0 2%	30 0 4%	47 0 8%	53 0 9%	57 0 9%
<i>hyrcanus</i>	5,206 46 7%	2,373 38 6%	1,209 18 1%	762 11 6%	294 3 3%	379 5 3%	1,308 24 6%	1,790 30 4%	1,848 22 7%	1,433 23 8%	1,841 29 9%	1,876 28 1%
<i>barburostris</i>	4,338 38 9%	3,712 49 8%	3,513 52 5%	1,661 25 2%	364 4 1%	173 2 1%	1,462 27 5%	2,439 41 4%	2,431 29 8%	1,472 24 4%	2,035 33 0%	3,131 47 9%
<i>pseudojamesi</i>	265 2 1%	51 0 7%	14 0 2%	28 0 4%	67 0 8%	181 2 6%	252 4 7%	381 6 5%	517 6 4%	681 11 3%	569 9 2%	432 6 5%
<i>varuna</i>	972 8 0%	379 5 1%	460 6 9%	230 3 5%	59 0 7%	21 0 3%	106 2 0%	970 16 5%	2,718 33 4%	1,979 32 8%	1,282 20 8%	907 13 6%
<i>aconitus</i>	324 2 7%	118 1 6%	162 2 4%	121 1 8%	20 0 2%	16 0 2%	15 0 3%	118 2 0%	363 4 5%	261 4 3%	210 3 4%	133 2 0%
<i>fuliginosus</i>	127 1 1%	267 3 6%	486 7 3%	584 8 9%	434 4 9%	367 5 2%	158 3 0%	56 1 0%	41 0 5%	69 1 1%	119 1 9%	97 1 5%
<i>philippinensis</i>	11 0 1%	10 0 1%	5 0 1%	5 0 1%	12 0 1%	6 0 1%	21 0 4%	20 0 4%	38 0 5%	36 0 6%	13 0 2%	22 0 3%
<i>culicifacies</i>	0	0	0	1 0 02%	0	1 0 01%	0	0	0	0	0	0
<i>tessellatus</i>	0	0	0	0	0	2 0 03%	3 0 06%	9 0 15%	33 0 41%	0	0	0

Statement of the total anopheles larvae collected during 1926-1930
Monthly total figures and average percentages

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
<i>subpurus</i> and <i>vagus</i>	556 1 7%	1,763 6 1%	7,669 21 7%	18,023 50 1%	26,640 74 4%	22,651 66 0%	9,781 35 3%	3 435 8 5%	1,966 4 4%	781 2 5%	721 1 5%	528 1 1%
<i>hyrcanus</i>	14,246 44 4%	9,935 34 2%	6 756 19 1%	4,905 13 6%	2,702 7 5%	4,172 12 2%	5,505 19 9%	10,155 25 0%	9,800 21 9%	7,403 21 5%	14,375 31 0%	15,778 34 0%
<i>barbirostris</i>	12,059 37 6%	13,331 45 9%	15,271 43 3%	7,068 19 3%	2,450 6 8%	2,577 7 5%	6,517 23 5%	12,037 29 6%	10,563 23 5%	6,945 21 5%	15,219 32 8%	18 167 39 2%
<i>pseudotomases</i>	1,202 3 7%	490 1 7%	297 0 8%	444 1 2%	610 1 7%	1,306 3 8%	1,406 5 1%	2,621 6 4%	2 511 5 6%	2 124 6 7%	2,157 4 6%	2 396 5 2%
<i>varuna</i> and <i>aconitus</i>	3,229 10 1%	2,392 8 2%	3,418 9 7%	3,295 9 1%	1 228 3 4%	1 158 3 4%	3 319 12 0%	11,514 25 4%	19 520 43 6%	13 823 43 5%	13 369 28 8%	8 763 19 0%
<i>fuliginosus</i> and <i>philippi- nensis</i>	811 2 5%	1,102 3 8%	1,823 5 5%	2,211 6 1%	2 097 5 8%	2,336 6 8%	1 007 3 6%	661 1 6%	370 0 8%	343 1 1%	527 1 2%	667 1 4%
<i>culicifacies</i>	0	26 0 00%	54 0 16%	74 0 21%	46 0 13%	57 0 17%	75 0 27%	22 0 05%	2 0 01%	0	0	0
<i>tessellatus</i>	0	0	0	0	0	27 0 08%	90 0 12%	181 0 45%	51 0 11%	16 0 05%	1	4 0 01%
TOTAL	32,103	29,039	35 288	36,010	37,773	34,284	27 703	40,626	44 783	31 838	46,369	46 283

THE ESCAPE OF THE FILARIA LARVA FROM THE PROBOSCIS OF *CULEX FATIGANS*

BY

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[Received for publication, September 14, 1931]

IN order to study the process of penetration of the skin by mature larvæ of *Wuchereria (Filaria) bancrofti* Cobbold, the authors fed some experimentally infected specimens of *Culex fatigans* Weid on a volunteer. After a short time, the mosquitoes were taken out to see if the *Filaria* larvæ had actually emerged from the proboscis. The mosquito was stunned by a shake, put on a slide and a small coverslip placed lightly on it to keep the mosquito from flying away if it should recover. While examining the proboscis of one of these mosquitoes under the microscope, a *Filaria* larva was observed to emerge from the extreme tip of the right labella of the mosquito. At first the extreme anterior end of the larva was seen projecting beyond the tip of the labella, and after some exertion the worm managed to emerge. When a quarter of its length was free, it rapidly made its way out without any difficulty. As soon as the worm larva showed itself well out of the tip of the labella, some Bles solution was added and the specimen was immediately killed with the fluid, by that time the worm larva had more than half emerged out of the proboscis. From the time the tip of the worm had showed itself well out of the labella to the time it was killed, was barely a few seconds. The emergence of the larva from the proboscis was very rapid and it was fortunate that the worm was killed before it had fully emerged.

After keeping the entire mosquito in Bles solution for one hour, the head of the mosquito was separated, stained with alcoholic eosin and mounted in Canada

balsam A photomicrograph of the proboscis with the half emerged filaria larva is reproduced in Plate LIII, fig 1

An examination of this preparation shows that the *Filaria* larva has emerged from the extreme tip of the labella and not through the point of junction of the labium with the labellæ through the so-called 'Dutton's membrane' Plate LIII, fig 2, is a more magnified photomicrograph of the tip of the proboscis and shows the larva partly inside the proboscis, partly within the labella and partly outside The point of emergence of the *Filaria* larva is very clearly demonstrated here and it is evident that this is the extreme tip of the labella

This observation on a natural finding of the larva in the process of emerging from the proboscis without any mechanical pressure or application of heat is interesting While examining infected *Culex fatigans* mosquitoes under the microscope the authors have observed how *Filaria* larvæ persistently attempt to penetrate the extreme tip of the labella When there is even the slightest disturbance, either increased temperature, or a little increased activity of the mosquito, as for instance, an attempt on the part of the mosquito to bite, or a little shaking of the mosquito, these larvæ are stirred to activity They then move about rapidly and try to pierce the extreme tip of the labella They have never been observed to attempt to pierce at any other point This has been confirmed on many subsequent occasions and it seemed evident from the manner in which *Filaria* larvæ try to penetrate through the extreme tip of the labella that they probably make their way out at that point The present finding of a *Filaria* larva killed while actually emerging through the tip of the labella confirms this conclusion

The manner in which *Filaria* larvæ emerge from the proboscis of the mosquito has been discussed by several previous workers Grassi and Noe (1900) thought that the larvæ escaped by a rupture of the dorsal groove of the labium when the mosquito feeds Annett, Dutton and Elliott (1901) thought that the larva made its way out by rupturing a membrane at the apex of the labium on its dorsal side at the junction of the labium and the labella, and this membrane has been called the 'Dutton's membrane' Lebrede (1905) and Fulleborn (1908) also confirmed Lebrede's findings These workers considered that *Filaria* larvæ made their way out through the membrane on the dorsal side of the labium at the point of its junction with the labella

It appears that Mochizuki (1910) was the first to demonstrate that *Filaria* larvæ emerged not through 'Dutton's membrane' but through the extreme tip of the labella This was confirmed by Yamada and Komori (1926) who by pressing on the tip of the labella allowed *Filaria* larvæ to escape through that point, they, however, could not observe larvæ escaping under natural conditions

While these observations were based on material which had been manipulated, one could raise the question as to whether the larvæ do actually effect their escape

through that point under natural circumstances. The present observations based on the emergence of the *Filaria* larva after attempting to bite man, is a finding of the escape of *Filaria* through the tip of the labella under natural conditions and confirms the conjectures of Yamada and Komori (1926) who based their conclusions on material in which the bursting of the tip of the labellæ was facilitated by mechanical pressure.

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EXPLANATION OF PLATE LIII

- Fig 1 Photomicrograph of the proboscis of *Culex fatigans* showing a larva of *W (Filaria) bancrofti* more than half emerged out of the proboscis (Magnification $\times 53$)
- „ 2 Photomicrograph of apex of the proboscis showing the point of emergence of the worm at the tip of the right labella (Magnification $\times 109$)
- „ 3 A more magnified view of the apex of the proboscis showing the tip of the right labella and the worm larva emerging out of it (Magnification $\times 224$)
- „ 4 Another picture of same showing the worm larva partly within the labium, partly within the labella and partly outside (Magnification $\times 224$)



Fig 1



Fig 2



Fig 3.

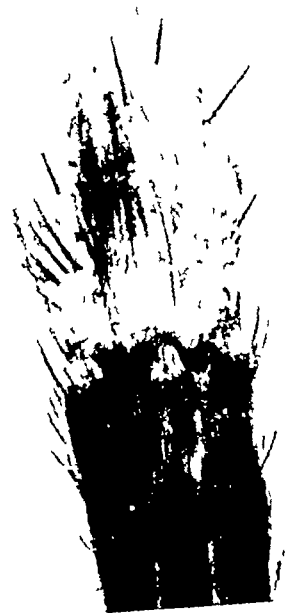


Fig 4

THE MALARIAL PIGMENT (HÆMOZOIN) IN THE SPLEEN

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[Received for publication, September 26, 1931]

It is universally admitted that malarial pigment (hæmozoïn) is an iron containing derivative of hæmoglobin and some even suggest its being identical with hæmatin. Castellani and Chalmers (1919) state that the pigment is soluble in alkali, insoluble in water, alcohol, chloroform ether, acids and that it contains iron in an organic form which will not give Berlin blue reaction. Manson-Bahr (1929) states that the pigment is insoluble in strong acids is only *altered* by potash, not dissolved as stated above, but is rapidly soluble in ammonium sulphide. Brown (1911) regards the pigment as identical with hæmatin owing to the similarity of solubility, spectroscopic properties and in containing iron. Mayer (1922) has demonstrated iron by Berlin blue reaction in subtertian malaria by allowing the pigment to be acted upon by acid alcohol, but admits that the reaction is capricious. Lignac (1924) tried unsuccessfully to reproduce Mayer's reaction and states that potassium ferrocyanide and hydrochloric acid, if remaining too long in contact with each other, will produce Prussian blue without any extraneous iron being present. Warasi (1927) states that the pigment is insoluble in water, alcohol, ether, chloroform, hydrochloric acid, *caustic soda* and slightly soluble in acetic acid and 'Saure alkohol'.

It will be observed that most of the assertions are in disagreement with each other and that there does not exist a specific test by which the presence of hæmozoïn may be determined. The following investigation was carried out to evolve a chemical reaction which might ultimately help in the diagnosis of malaria. Owing to the lack of knowledge of their chemical nature, a satisfactory classification of

the protozoal and bacterial pigments does not exist. Most of these pigments have been put into various categories according to their solubility in various solvents, few of them, however, giving specific chemical reactions, e.g., carotinoids, anthocyanines, melanins, etc. Following the usual methods employed, we have studied the subject under the following headings —

- 1 Solubility in various solvents
- 2 Spectroscopic studies of the solutions of the pigment
- 3 Attempts to test the presence of iron
- 4 Flocculation and complement deviation tests

1 SOLUBILITY IN VARIOUS SOLVENTS

The effects of solvents have been studied mainly on the sections of malarial spleens, as blood films are either dissolved or altered by the effect of the various chemicals. The technique employed is as follows —

To harden the tissue, suitable portions of the spleen were left in formalin solution (10 per cent) for a few days, then successively passed through 50 per cent, 70 per cent, 90 per cent and lastly absolute alcohol. After treatment with the latter, the tissue was transferred to xylol and embedded in paraffin. The sections were treated with xylol to remove the paraffin, washed with absolute alcohol and finally with distilled water. The sections were covered with the chemicals to be tested either pure or in suitable dilutions, the effect was noted from time to time under the $\frac{2}{3}$ and $\frac{1}{6}$ objectives. The following observations were made with the undermentioned reagents —

(a) *Alkalis* — It was found that the pigment was soluble in N/1 sodium hydroxide, liquor potassi B.P., liquor ammonia fortis B.P. On addition of alkalis, within 5 to 15 minutes, depending on their concentration, the dark granules become deep brown in colour, develop a yellowish brown haze around themselves and ultimately dissolve away. If the section is now washed with distilled water, it is found to be devoid of any pigment. The time required to dissolve the pigment with liquor ammonia fortis is usually half an hour. To test further the minimum dilutions of alkali required for solution of the pigment, sections were treated with various dilutions of sodium hydroxide and it was found that dilutions weaker than N/20 sodium hydroxide had no effect.

It has been asserted that this pigment is merely altered, and not dissolved in the alkalis, to investigate this question the following experiment was carried out —

Two sections were treated with N/1 sodium hydroxide for 12 hours, one of these was washed with distilled water to get rid of the alkali, while in the case of the other the alkali was neutralized with N/1, sulphuric acid, and an excess of acid left for 15 minutes and then washed away. Both sections were stained with

hæmatoxylin and Biebrick-scarlet, together with an ordinary untreated malarial spleen section to act as a control. It was noted that the former two sections showed no trace of pigment, while the control did, proving that in case of alkalis there is no question of alteration but perfect solution. The acid in the second experiment was added to neutralize the alkali and so reconvert the 'altered' pigment, but without success.

(b) *Acids* —The pigment was insoluble in acids, even in strong nitric, hydrochloric and sulphuric acids, no alteration in the appearance of the pigment being noted. It showed no signs of dissolving or even becoming fainter after 24 hours' treatment with alcohol acidulated with acetic or other acids (*vide* reference to Mayer's work in the beginning of this paper).

(c) *Fat solvents and other miscellaneous chemicals* —The pigment was found to be insoluble in ether, chloroform, xylol, benzine, acetone, absolute alcohol, acid alcohol (5 per cent glacial acetic acid), carbon bisulphide, liquor formaldehyde, amyl alcohol, glycerine or carbon tetrachloride. Strong solutions of ammonium sulphide or sodium chloride did not produce any change neither did antiformin, silver nitrate (1 per cent), or strong solution of bleaching powder. Hydrogen peroxide requires a special mention as a prolonged treatment (24 hours or so) with this reagent causes a bleaching of the pigment. The granules are still visible but there is no colour. It was not possible to restore the colour with various oxidizing or reducing agents.

2 SPECTROSCOPIC STUDIES

(1) Having ascertained that the pigment is soluble in alkali and presuming it to be identical with hæmatin (Brown, 1911), the following procedure was adopted for spectroscopic study —

(a) A solution of N/1 sodium hydroxide was added to a finely minced sample of malarial spleen (4 l) and the mixture was left for 12 hours at room temperature.

(b) A similar procedure was adopted using a non-malarial human spleen, i.e., normal spleen, to act as a control.

It was found that solution (a) was a clear greenish fluid, while solution (b) was straw coloured. No absorption band was noticed in either solution as such or when diluted suitably but on addition of ammonium sulphide a sharp band was noticed, starting at the yellow and extending to the green in both cases. That this spectroscopic phenomenon is non-specific is evident from the similar results obtained from the control.

(2) *Blood* —(a) A sample of malarial blood (pigmented parasites B.T. seen in abundance) was hæmolyzed by addition of distilled water, the hæmoglobin was removed by repeatedly centrifugalizing and washing the deposit with distilled water.

(b) A sample of non-malarial blood was dealt with as above to act as control

The deposits were dissolved in N/10 sodium hydroxide and examined spectroscopically. No absorption bands were noticed except on addition of an equal part of ammonium sulphide, when a band similar to the one noticed under experiment (1) was seen in both cases

(3) *Urine*—Presuming that some of the malarial pigment would be excreted in the urine, a sample of the above case, *see* (2), was obtained. It was centrifuged and the deposit washed several times to get rid of the traces of urine. The deposit was dissolved in N/1 sodium hydroxide and examined spectroscopically, but no absorption band was noticed even on addition of ammonium sulphide

3 ATTEMPTS TO TEST THE PRESENCE OF IRON

Having established that the pigment is soluble in alkalis the following procedure was adopted to test the presence of iron —

A spleen section showing a profusion of the pigment was focused under the $\frac{2}{3}$ objective of the microscope and then flooded with N/10 sodium hydroxide. When the pigment had turned brownish and a yellowish haze was noticed around the individual granules, the alkali was pipetted off and N/10 hydrochloric acid run on the section. It was intended to test for the presence of iron after acidifying, by ammonium sulphocyanide solution but it was noticed that the section had turned reddish brown, the pigment masses being of a deeper rusty red colour, hence the reagent was discarded, and potassium ferrocyanide solution was substituted. The whole section turned blue but the pigment masses still retained the rusty red colour

Though the presence or absence of iron could not be precisely settled we performed the following experiments to ascertain if the reaction, *viz* the turning of the pigment yellowish brown, on addition of alkali followed by a rusty red colour on addition of acid was specific —

(1) Seven specimens of malarial spleen were obtained from out-stations (one from the Medical College, Rangoon, one from Haffkine Institute, Bombay, four from the School of Tropical Medicine, one from a local military hospital). The technique followed was the same as described above, and all the sections, except the one from Bombay, showed coarse black pigment masses. The latter exception showed fine dark brownish granules which disappeared rapidly on addition of alkali. This section had to be dealt with specially, the alkali being washed off after the lapse of a minute or so followed by addition of the acid. Owing to the minute size of the granules the reaction, though definitely positive under the $\frac{1}{6}$ was not so well marked under the $\frac{2}{3}$ objective. In all the other sections, the addition of the alkali N/10 sodium hydroxide turned the granules brownish with a yellowish haze around them, which was transformed into a rusty red colour on addition of

acid (N/10 hydrochloric acid) A similar reaction was noted in sections of the malarial liver though not so well marked

(2) Kala-azar spleen was dealt with in a similar way to the malarial spleen but the above specific reaction was not noted

(3) Skin sections showing marked deposit of melanin pigment grew fainter on addition of alkali but turned black again on addition of acid

This test was inapplicable to blood films as the N/20 sodium hydroxide being the minimum required for dissolving the pigment, dissolved the film *en masse*. Specimens of blood from malarial patients showing marked pigmentation in the infected red cells were hæmolyzed and all traces of hæmoglobin were removed by washing repeatedly with distilled water. The deposit thus obtained consisted of fine granules which dissolved in alkali too rapidly to permit the addition of acid. The same was true in the case of the samples of urine from malarial patients dealt with as described under 'spectroscopic examinations'.

4 FLOCCULATION AND COMPLEMENT DIVIATION TESTS WITH ALKALINE SPLEEN EXTRACT

Owing to the solubility of hæmoglobin and other constituents of a malarial spleen in sodium hydroxide solutions the possibility of this extract acting as an antigen was contemplated, and a brief summary of the technique followed and the results obtained is given below —

(1) A portion of malarial spleen was finely minced and an equal volume of N/1 sodium hydroxide was added and left for 12 hours at room temperature. The tissue extract thus obtained was filtered and used as follows —

(a) One c.c. of the above extract was mixed with 0.1 c.c. of serum of a distinct case of malaria (parasites seen in a blood film). The quantity of serum in question was set up with 1 c.c. of N/10 sodium hydroxide to act as a control.

(b) The alkaline spleen extract was neutralized with N/1 hydrochloric acid and similar quantities put up as in (a). In both cases normal sera from a non-malarial case was put up as control. The tubes containing the sera and extract were incubated at 55°C and then examined for flocculi with a hand lens (magnification 20). There were altogether ten tests put up from malarial cases in whose blood B.T. parasites had been seen, the results in all cases, including the controls, were negative.

(2) In the complement deviation test the technique followed was similar to the one used in this laboratory for the Wassermann reaction for syphilis (Medical Research Council Method No. 1). The malarial antigen was neutralized spleen extract similar to the one described under (1) (b).

The number of sera and controls tested in this way were ten from the same cases tested under (1), but the results were negative in the malarial as well as the control tubes. Owing to the negative results a detailed description of the tests has not been given.

SUMMARY

1 The malarial pigment deposited in the spleen is soluble in alkalis and insoluble in acids, usual fat solvents and various other reagents described. It is bleached by hydrogen peroxide solution when kept in contact for a long time.

2 Spectroscopically the alkaline solution of the pigment does not produce absorption bands of alkaline hæmatin, in fact nothing specific is seen in the spectrum. It may be added that the alkaline solution when suitably dealt with does not produce the crystals of hæmatin hydrochloride.

3 On addition of an alkali the malarial pigment in the spleen turns brown with a yellowish haze around it. On substituting an acid for the alkali the granules of the pigment turn rusty red. We regard this test as specific for hæmozoin in the tissues, but may state that the number of tissues we have examined have been too few to make a positive assertion.

4 The alkaline solution of the minced spleen by itself or when neutralized is incapable of acting as an antigen for flocculation or complement deviation tests.

ACKNOWLEDGMENTS

We would like to record our thanks to Lieutenant-Colonel H. W. Acton, C.I.E., I.M.S., Director, School of Tropical Medicine and Hygiene, Calcutta, who went through the manuscript of the paper and confirmed the solubility reaction mentioned above. We would, also, take this opportunity of thanking other Medical Officers, who supplied us with the material.

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ON THE DISTRIBUTION OF PROTECTIVE PRINCIPLE IN DIFFERENT PROTEIN FRACTIONS OF HORSE SERUM IMMUNIZED AGAINST SNAKE VENOM

BY

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[Received for publication, September 30, 1931]

DURING recent years successful methods have been employed to concentrate and purify therapeutic sera and their value in treatment has been justified by clinical experience. The presence of antibodies in the immune serum does not make any appreciable change in its chemical composition as compared with normal serum although their presence is demonstrated by its power of neutralizing the specific toxin.

It was discovered that the antibodies could be precipitated from immune sera by various salts along with a certain fraction of serum proteins and this fact has been utilized to get rid of useless non-specific proteins and issue a more or less purified product for therapeutic use.

Banzhaf (1928) with his comprehensive experience on this subject has stated that in all the horse immune sera most of the antibodies are in combination with the pseudoglobulin fraction of the serum proteins and he also admits of variability of its location in different animals. As to the nature of this antibody-pseudoglobulin complex very little is known. More recently Frankel and Olitski (1931) have reported their capability of obtaining antibodies from immune sera free from any proteins whatever, quoting the negative results of biological and chemical test for proteins in their antibodies. As we do not, at the present stage of our knowledge, exactly know the nature of the antibodies, we are satisfied if their presence in concentrated and purified sera is confirmed by observations *in vitro* and *in vivo*.

If the work of the last mentioned authors is confirmed and a suitable technique evolved to lay our hands on antibodies in such a pure state serum therapy would have made a marvellous stride

Kasauli antivenene manufactured from horses is extensively used throughout this country with very good results. That purification and concentration of antivenene is desirable needs no reasoning when we come to think of the possible amount of venom a healthy adult cobra or Russell's viper can inject at a bite and the enormous quantities of natural antivenene required to neutralize it (Byam and Archibald, 1921). The justification of such a step has been very ably mentioned by Acton and Knowles (1915) who took up the subject in 1915 and came to the conclusion that the antibodies in antivenene are in combination with serum globulins and can be precipitated from the immune goat serum by 40 per cent saturation with ammonium sulphate. This was further confirmed by Caius, Iyengar and Anderson (1924) who, however, deprecated the method as they could only get 'Concentration with loss of potency'. In view of such conflicting results it was thought desirable to re-investigate the problem with improved technique as the actual distribution of the antibodies has to be considered when concentration is desired in order to enable one to eliminate the non-effective protein material without losing any antibodies.

These experiments were conducted as a preliminary step towards concentration and to find out —

- (1) How the antibodies are actually distributed in the antivenene raised from horses?
- (2) Whether they can be recovered without much loss after useless proteins have been discarded?

Several methods of chemical precipitation by salts of ammonium, magnesium and neutral salts of heavy metals followed by dialysis were employed to separate out the different protein fractions but finally the fractional precipitation of antivenene by ammonium sulphate was found to give encouraging results.

Each protein fraction of the antivenene obtained by various methods was tested for its antibody content against cobra venom according to the method employed to standardize antivenomous serum manufactured at Kasauli (Anderson and Caius, 1925).

EXPERIMENTAL OBSERVATION

1 *Euglobulin fraction*

Preparation —

A Euglobulin is insoluble in acidulated distilled water and falls down as a precipitate. Taking advantage of this fact, to one part of antivenene 9 parts of distilled water were added and the mixture thoroughly shaken and allowed to

sediment The precipitate of euglobulin was collected, dissolved in minimal quantity of normal saline solution and tested against cobra venom on pigeons

B 40 c c of antivenene were directly dialysed in running tap-water for 4 days and then for 2 days in distilled water (Morgan and Fairbrother, 1930) A considerable portion of euglobulin was thrown down as precipitate during dialysis The precipitate was separated by centrifuging and after washing thrice with distilled water tested against cobra venom on pigeons

C To 100 c c of antivenomous serum was added an equal volume of saturated solution of ammonium sulphate and the mixture allowed to stand overnight after thorough shaking A heavy precipitate of pseudoglobulin and euglobulin was obtained After filtration the precipitate was shaken in 100 c c saturated solution of sodium chloride and undissolved euglobulin fraction filtered off (Morgan and Fairbrother, 1930) It was then dialysed free of salts and dissolved in 10 c c of normal saline solution and tested against cobra venom on pigeons

D A further specimen of this fraction was obtained by salting the serum out with ammonium sulphate according to a method described by Max Stumia and others (1930) and as this method proved very successful, a brief account of this is added and it will be referred to for the sake of abbreviation as the 'ammonium sulphate method'

Serum without preservative is diluted with an equal volume of 0.85 per cent normal saline solution To one volume of serum an equal volume of 66 per cent saturated solution of ammonium sulphate is added, the mixture allowed to sediment for 15 minutes and filtered This separates the 'euglobulin fraction' To the filtrate enough saturated ammonium sulphate solution is added to bring the concentration to 50 per cent This precipitates down the pseudoglobulin fraction The filtrate is then saturated with ammonium sulphate and now the albumen falls down as a precipitate All the precipitates were dialysed for 4 days in the ice box (our experiments were carried out at room temperature) and when ammonia free (as tested by Nessler's reagent) examined for antibodies both qualitatively and quantitatively

None of the euglobulin fractions prepared by methods A, B, C and D showed any antibodies when tested against cobra venom on pigeons

2 *Pseudoglobulin fraction*

Preparation —

Mainly the ammonium sulphate method was employed to obtain this fraction and the resulting precipitate dialysed free of ammonia Different workers recommend different concentrations of salt for separating this fraction, but about 50 per cent saturation of serum after removal of euglobulin brings down all the pseudoglobulin in immune horse serum

This was the only fraction which always showed the presence of antivenene principle when its neutralizing power against cobra venom was tested on pigeons It was rather encouraging to find that all antivenene principle of the serum could be recovered in this fraction without any loss either in quality or quantity Samples

of antivenene from various horses were studied similarly and each of them confirmed the results of the sample experiment quoted below —

TABLE

Comparing the neutralizing power of pseudoglobulin fraction of antivenene with a sample of natural antivenene used for obtaining the pseudoglobulin

Sample of natural antivenene	Total potency expressed in mg of cobra venom	Pseudoglobulin fraction separated	Total potency of pseudoglobulin fraction expressed in mg of cobra venom
40 c c Brew No 149 V 32A 8 9 30 Potency 1 c c = 0.5 mg	20 mg	34 c c Potency 1 c c = 0.6 mg	20.4 mg

3 Albumen fraction

Preparation —

This fraction obtained by ammonium sulphate method after separation of euglobulin and pseudoglobulin was dialysed and tested. No protection to pigeons was afforded.

4 *Filtrate* obtained after separation of different protein fraction was dialysed and tested. No protection was afforded to pigeons.

Immune sera differ very little in composition from the normal sera although it is stated that the pseudoglobulin fraction increases with immunization (Kirkbride and Murdick, 1927).

Further, it has been stated that although there are variations in the ratios of globulin and albumen in the sera of different individuals, generally the ratio of globulin to albumen is always less than unity. The albumen therefore forms more than 50 per cent of serum proteins and having no therapeutic value it is worth while to discard this fraction with advantage, but out of the globulins again the euglobulin fraction can also be discarded. This leaves pseudoglobulin the only protein forming less than 47 per cent of the total protein of horse's serum with all the antivenene principle with it (Robertson, 1924).

Experiments with regard to preparation on a large scale, storage and sterilization of the purified antivenene are in progress and a further communication will be made.

Our thanks are due to Colonel Sir S R Christophers, *Kt*, *CIE*, *OBE*, *FRS*, *IMS*, Director, Central Research Institute, Kasauli, for his sympathetic criticisms and encouragement in the study of this problem

We are also indebted to Mr N D Kehar, *MSc*, Chemist of Malania Survey of India, for giving useful suggestions on the practical side of the technique employed for separating the different protein fractions

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A COMPARATIVE STUDY OF THE EFFICIENCY OF CHOLERA
VACCINE STORED IN A 'FRIGIDAIRE' AT 4°C
AND IN A BIOLOGICAL INCUBATOR
AT 37°C

BY

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[Received for publication, October 1, 1931]

EXPERIMENTS described in this communication were designed to determine the effect of age and temperature on the antigenic value of cholera vaccine

Owing to the difficulty associated with the actual production of this disease in experimental animals and the debatable part played by complement deviating bodies, hæmolysins, opsonins, etc., as defensive factors in a serum, we have used agglutino-genic response as the index of antigenic potency

Cholera vaccine six months after the date of manufacture is considered as time expired and unfit for prophylactic use. Large quantities of unexpended vaccine have consequently to be condemned and destroyed at the source of manufacture. Nowadays when frigidaire's are considered as a more or less essential equipment of laboratories and vaccine depots, it was thought advisable to investigate the effect of storage at low temperatures on the potency of cholera vaccine. Comparative tests were carried out with the same brew of vaccine stored at 37°C, a temperature which probably corresponds more than favourably with the room temperature of most places in the plains of India and of tropical countries generally. The cholera vaccine employed was a killed and carbolized suspension of 8,000 million *V. cholerae* per c.c. The animals used for the immunity experiments have

been large healthy rabbits of both sexes free from any visible signs of disease. They were injected subcutaneously with 0.5 c.c. and 1 c.c. doses at weekly intervals and their sera tested for agglutinin content 12 days after the administration of the last dose. Thus the doses of vaccine used were the same as are employed for prophylactic purposes in man. Four animals were used on each occasion, two for the trial of the vaccine stored in the frigidaire and the other two for the vaccine kept at 37°C.

The following tabulated results show in detail the agglutination titre of rabbit sera immunized in the manner described above —

TABLE J

Cholera vaccine freshly prepared

Rabbit number	Weight in grammes	Agglutination results								Mean titre
		10	20	40	80	160	320	640	1,280	
R1	1,650	+	+	+	+	+	0	0	0	1 200
R2	1,440	+	+	+	+	+	0	0	0	
R3	1,500	+	+	+	+	+	+	0	0	
R4	1,310	+	+	+	+	+	0	0	0	

+ = Agglutination
0 = No agglutination

DISCUSSION

It has to be remembered that we are dealing throughout these experiments with agglutinin response as a criterion of antigenic efficiency of the vaccine. This may bear little or no relation to immunity as defined by the ability of inoculated animals to resist infection after a dose of live culture of organisms lethal for uninoculated animals. Secondly, due allowance has to be made for variation in response to vaccine administration shown by the experimental animals.

TABLE II

Cholera vaccine stored at 4°C in the refrigerator

1—6 MONTHS OLD										6—12 MONTHS OLD									
Age of vaccine	Rabbit number and weight in grammes	Agglutination results								Age of vaccine	Rabbit number and weight in grammes	Agglutination results							
		10	20	40	80	160	320	640				10	20	40	80	160	320	640	
1 month	R29 1,100	+	+	+	+	+	0	0		7 months	R41 1,230	+	+	+	+	0	0	0	
1 month	R30 1,230	+	+	+	+	+	0	0		7 months	R42 1,290	+	+	+	+	0	0	0	
2 months	R31 1,430	+	+	+	+	0	0	0		8 months	R43 1,275	+	+	+	+	+	+	+	+
2 months	R32 1,325	+	+	+	+	0	0	0		8 months	R44 1,165	+	+	+	+	+	+	0	0
3 months	R33 1,400	+	+	+	+	+	+	+		9 months	R45 1,350	+	+	+	+	+	+	0	0
3 months	R34 1,550	+	+	+	+	+	+	0		9 months	R46 1,400								
4 months	R35 1,440								Died during experiment	10 months	R47 1,340	+	+	+	+	+	+	+	0
4 months	R36 1,860	+	+	+	+	+	+	0		10 months	R48 1,070	+	+	+	+	0	0	0	0
5 months	R37 2,490	+	+	+	+	+	0	0		11 months	R49 1,250	+	+	+	+	+	0	0	0
5 months	R38 2,010	+	+	+	+	+	0	0		11 months	R50 1,360	+	+	+	+	+	0	0	0
6 months	R39 1 250	+	+	+	+	+	+	0		12 months	R51 1,300	+	+	+	+	+	0	0	0
6 months	R40 1,330	+	+	+	+	+	+	0		12 months	R52 1,350	+	+	+	+	+	0	0	0

TABLE III

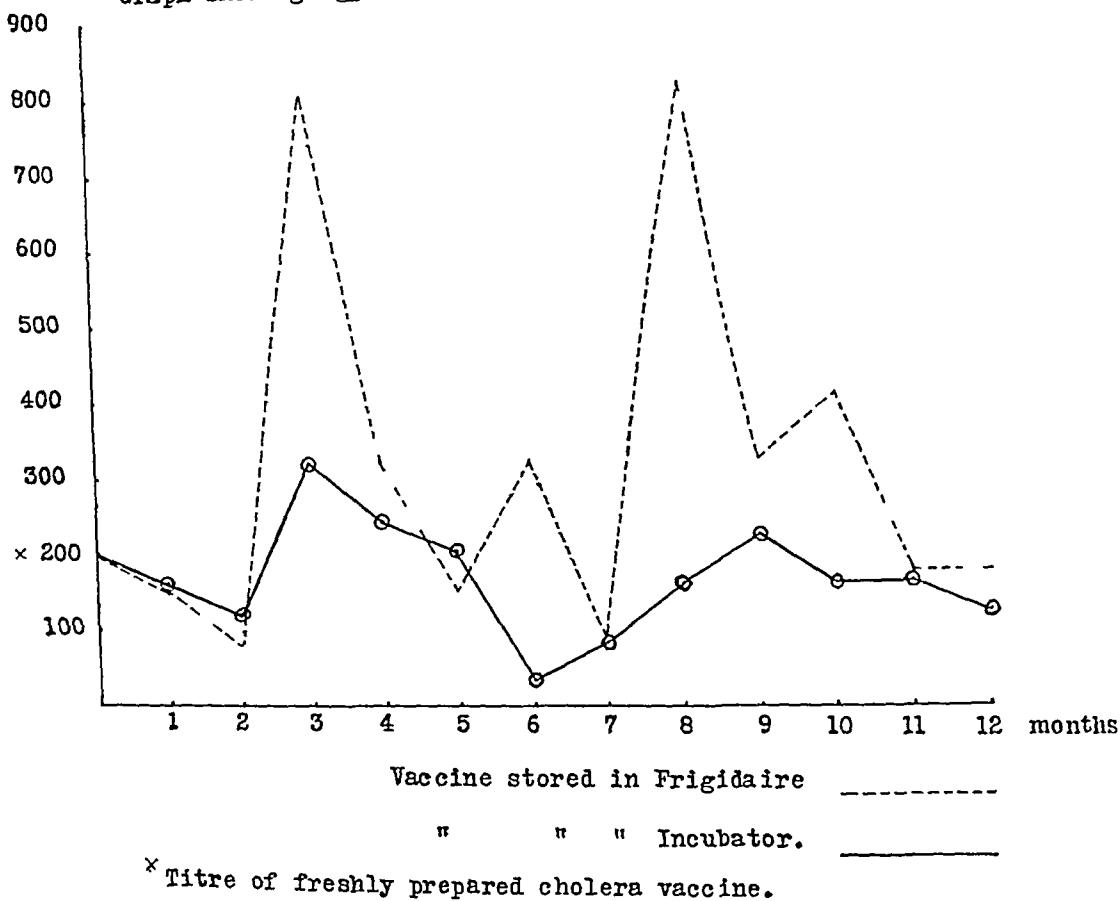
Cholera vaccine stored at 37°C

1--6 MONTHS OLD													6--12 MONTHS OLD												
Age of vaccine	Rabbit number and weight in grammes	Agglutination results								Rabbit number and weight in grammes	Age of vaccine	Agglutination results													
		10	20	40	80	160	320	640	1,280			10	20	40	80	160	320	640	1,280						
1 month	5 1,190	+	+	+	+	+	0	0	0	7 months	17 1,440	Died during experiment													
1 month	6 1,010	Died during experiment								18 1,580	7 months	18 1,580	+	+	+	+	+	+	+						
2 months	7 1,380	+	+	+	+	+	0	0	0	8 months	19 1,200	+	+	+	+	0	0	0							
2 months	8 1,180	+	+	+	+	0	0	0	0	8 months	20 1,340	+	+	+	+	0	0	0							
3 months	9 1,350	+	+	+	+	+	+	0	0	9 months	21 1,300	+	+	+	+	0	0	0							
3 months	10 1,530	+	+	+	+	+	+	0	0	9 months	22 1,350	+	+	+	+	+	0	0							
4 months	11 1,500	+	+	+	+	+	+	0	0	10 months	23 1,350	Died during experiment													
4 months	12 1,380	+	+	+	+	+	0	0	0	10 months	24 1,130	+	+	+	+	0	0	0							
5 months	13 2,060	+	+	+	+	0	0	0	0	11 months	25 1,380	+	+	+	+	0	0	0							
5 months	14 2,770	+	+	+	+	+	+	0	0	11 months	26 1,270	+	+	+	+	0	0	0							
6 months	15 1,415	+	+	0	0	0	0	0	0	12 months	27 1,400	+	+	+	0	0	0	0							
6 months	16 1,250	+	+	+	0	0	0	0	0	12 months	28 1,150	+	+	+	+	0	0	0							

From the analysis of the tabulated results it would appear that a temperature of 37°C does not cause any appreciable deterioration in the agglutinogenic power of the vaccine. Agglutination titre of freshly prepared vaccine—administered within about a week of its preparation—is about the same as that of one year old vaccine stored under above experimental conditions.

GRAPH

Graph showing agglutination curves.



Storage at a low temperature, as for instance that obtained in a frigidaire, is more efficient than in the incubator, but special precautions for preservation of vaccine are unnecessary at least for a year at a temperature not exceeding 37°C .

At present the life time of cholera vaccine stored at room temperature has been fixed for 6 months. From the observations noted above it would appear that

this period may be extended to a year. Before coming to such a decision, however, it has to be considered that the cholera vibrio in finished vaccine undergoes progressive autolysis and in this process may liberate its endotoxins in the medium. A vaccine stored for a period of more than 6 months may, therefore, give rise to unpleasant local and general reactions, making the practice of inoculation unpopular to the masses.

THE DIFFRACTION (HALOMETRIC) METHOD OF DETERMINING THE AVERAGE DIAMETER OF RED BLOOD CORPUSCLES.

BY

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[Received for publication, October 8, 1931]

Introduction.

OUR attention was drawn to this method through papers by Dr Lucy Wills (1930) dealing with the anæmia of pregnancy in Bombay and by McIver and Ghosh (1931) giving halometric readings in Indians. The method, if reliable, appeared to us to have applications not only in the study of anæmias, etc., but in the laboratory in the case of hæmolytic mixtures and in other conditions where it is desired to measure changes in the size of the red cell.

An important point seemed to us to be that only *dried* blood films had so far been used. In the drying of films, viscosity, surface tension of the plasma and possibly other factors might be supposed quite considerably to alter the size of the cell. Having found that an excellent diffraction image could be obtained with red cells in serum or other fluids we began a study of the method. The present paper gives a general account of the method with description of a technique by which readings can be taken in a simple way under the microscope.

Historical.

Thomas Young (1773-1829) was probably the first to use the principle of diffraction in the measurement of wool fibres and, later, of the diameter of red blood

corpuscles The instrument he used is referred to in textbooks on physics (Preston, 1928) under the heading of 'Young's Eriometer' (from Gr *Erion* = wool) It consists of an oblong metal plate in the centre of which is bored a small circular hole about 2 mm, or less, in diameter Surrounding this central aperture, and at a distance of about 2 cm radius, is a circle of about 32 pin-point holes This plate is set up in front of a strong light and, on viewing the light through a blood film, a series of concentric diffraction rings is seen round the central aperture, while the ring of pin-point holes appears as a circle of luminous points The film is moved towards the light, or away from it, along a graduated scale, until the circle of luminous points coincides with a selected coloured ring The distance of the blood film from the plate (previously determined for 'normal' or standard films) varies directly as the average diameter of the red blood corpuscles Since 1924 further advances in the use of this diffraction method have been made, more particularly in the study of pernicious anæmia and other pathological blood conditions Price-Jones (1910, 1911, 1920, 1921), for instance, studied the size of red blood corpuscles in pernicious anæmia by drawing the outline of 500 cells, in a dried film, magnified 1,000 times, and measuring them with a micrometer scale Ponder (1922) studied the changes in size and shape of the red blood corpuscles while undergoing hæmolysis by means of photography The amount of work involved in these methods, however, precludes them from being used for clinical purposes

Piper (1924) introduced a new diffraction method in which an ordinary dried blood film is used as a diffraction grating and the average diameter of the red blood cells deduced from the size of the coloured rings thrown on to a screen The method, however, required the use of a dark room and, later, Piper (1929) described a simpler and more perfect apparatus in the use of which a dark room was not necessary This consists of a wedge-shaped wooden or metal box about 40 cm long and 25 cm high In the narrow end of the box a lens is fixed and in the wide end a ground glass screen is placed in the focal plane of the lens The box is divided into two lateral halves by a vertical partition which extends from the back of the lens up to the screen In front of the lens is placed an opaque screen in which are two holes each 1.5 cm square In front of one hole is placed a normal blood film and in front of the other the film to be examined

By this method a normal blood film can be compared on the screen with a film of blood from a patient It is claimed that the average diameter of nearly a million corpuscles can be calculated with an accuracy of about one-tenth of a micron The formula used in the calculation of the diameter of the cells will be discussed in a later section of the paper

Edwards (1929) has simplified this apparatus considerably The source of light (a flash lamp of the 'pointolite' variety) and the collimating lens are

contained in one smaller box and the focusing lens and screen in a larger box, both boxes being fixed on a common base board. The apparatus is thus self contained and, as the author remarks, could be made more portable by making both boxes of the collapsible bellows type.

Another type of instrument, called a 'Halometer', which depends on the same principle and differs only in details of construction, is described by Eve (1929). A tiny electric bulb and a battery are enclosed in a small black box with a hole at one end against which the slide is clamped. The coloured halo seen on looking through the slide is duplicated by reflection from a pair of inclined mirrors and by moving a knob at the side of the box the two haloes are moved towards each other until their red edges are just touching. A scale, along which the knob moves, now records the angular measurement of the halo and, by reference to a printed table, this figure can be converted into one giving the average size of the red cells.

Emmons (1927) and Merlin Pryce (1929) described an apparatus depending on the principle of Young's original eriometer. The 'Clinical Eriometer' described by Emmons is compact and portable and consists of two telescopic tubes, one contains the source of light in front of which the perforated disc is fixed, the other tube fits into the former and carries the slide for the film. The instrument is calibrated for a definite ring of the halo and, when the tubes are moved till the circle of small holes in the disc coincides with this ring, the diameter of the red cells can be read from a scale fixed to the side of the instrument.

Merlin Pryce described two simple methods of arriving at the size of red cells. One is Young's eriometer as described in a previous paragraph. The other method is even simpler, the only apparatus required being two electric bulbs. These are placed about two feet apart at right angles to the line of sight. On viewing these two lights through a blood film, two haloes are seen, one from each light. These haloes appear to become smaller to the observer on approaching the lights, and larger on stepping back from the lights. The distance from the light at which any pair of corresponding coloured rings makes contact is directly proportional to the average size of the red cells.

Apparatus.

Finding the original methods not quite suited to our requirements, for one reason being rather bulky and, for another, not very suitable for observing red cells in fluid, we set about seeing whether some ordinary *microscopical* adjustment could be made so that examination by the diffraction method could be carried out more or less as one makes other observations on blood cells in the laboratory. Eventually we devised the very simple apparatus shown in Fig 1.

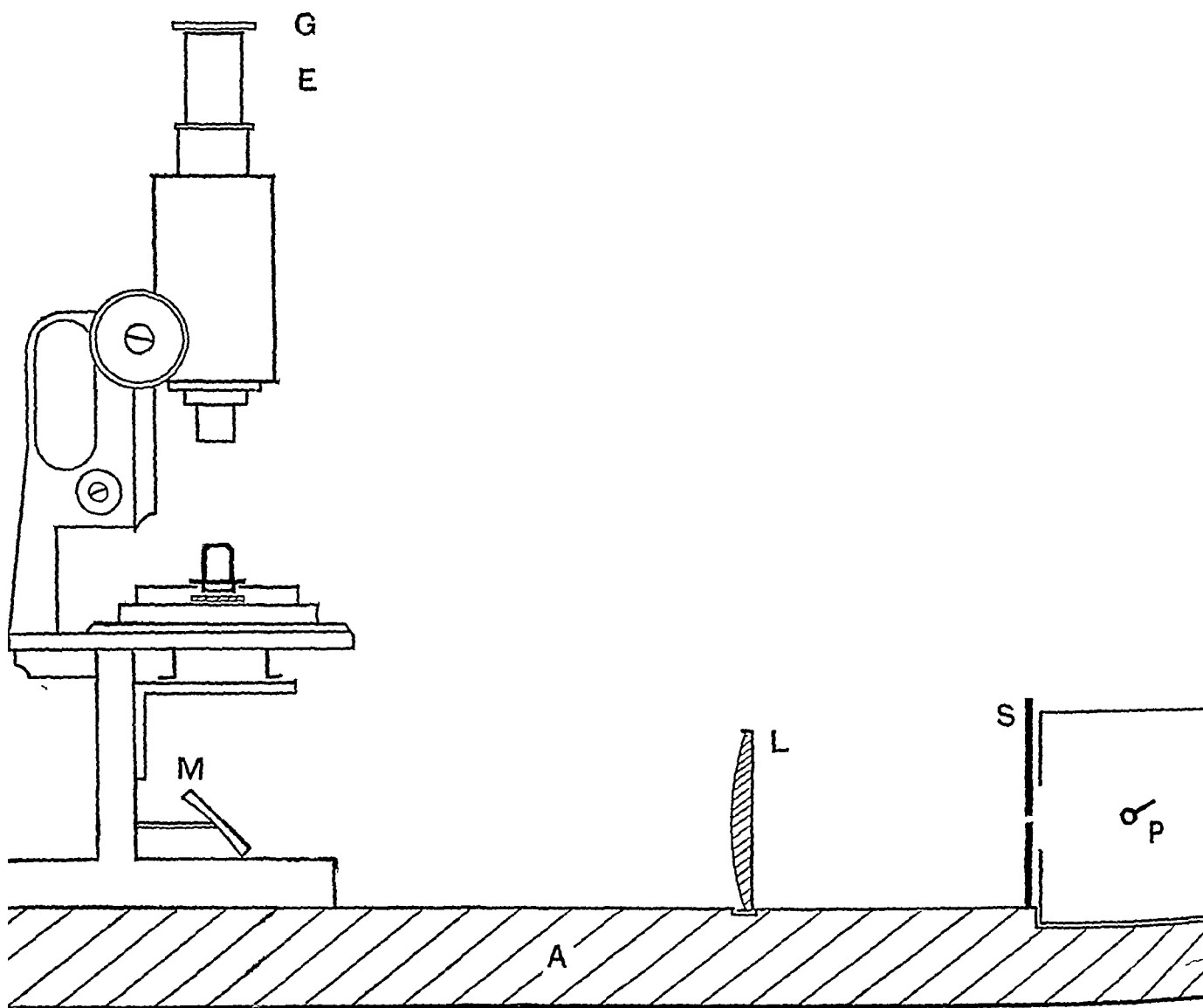


Fig 1

Showing the arrangement of the apparatus

- P —The source of light
- S —The screen
- L —The collimating lens placed at its focal distance from P
- M —Plane mirror and microscope, details of which are shown in fig 2
- E —No 1 eyepiece fitted with micrometer
- G —Smoked glass or portion of the dark part of a developed photographic plate used as required to diminish glare from the central disc
- A —Base board on which the apparatus is fixed

The essential part of the apparatus is a parallel beam of light, and this requires only a suitable source of light and a lens (an ordinary plano-convex is usually recommended) fixed at its focal distance from the source of light. A small opening of about 2-3 mm in an opaque screen placed between the light and the lens gives improved definition in the diffraction image. The parallelism in the beam of light can be checked by comparing the size of the disc just beyond the lens with that on a distant screen. It is convenient to have some sort of base board on which these parts can be permanently fixed and which will also take the microscope base in a fixed position, the distance between the microscope and the collimating lens is immaterial. For light we have found an 'Ediswan' 100 C P 'pointolite' to be very suitable.

In our earlier experiments we used various types of lenses to focus the diffraction image on to a screen. The focal distance of such lenses was determined and a ground-glass screen placed in the barrel of the microscope in the focal plane of the lens. Eventually it was found to be more convenient to use a suitable low power microscope objective (reversed over the preparation) and to observe the spectrum with another low power objective used in the ordinary way, the eyepiece of the microscope being fitted with an eyepiece micrometer by which the value of 'r' could be read. The apparatus is sufficiently explained in Fig 2.

To make an observation the lens L (Fig 2) is placed in position and some distant object is brought to a focus (as seen by looking through the microscope). The microscope is now set and should not be disturbed or focused in subsequent observations.

So set the microscope is now replaced on the base board, if it has been taken from this, and the preparation to be examined is placed under the lens L (Fig 2).

The spectrum can now be seen on looking down the microscope. If necessary, adjustment of the lens (L, Fig 2) can be made by simply moving it about by hand until the optical system is centred. The radius 'r' of the yellow ring of the second spectrum, for example, can now be read off on an eyepiece micrometer which has been previously calibrated against a stage micrometer.

Any two objectives can be used, for the collecting and examining systems respectively, that give the necessary magnification to display the spectrum, but this requirement, together with the limiting effect of the numerical aperture, practically limits the choice of lens to two or three sizes. We have found a combination consisting of a Zeiss objective AA 12 of 0.3 n.a. (focal length, 15.6 mm) as a 'collecting' lens and an objective a_2 of magnification 3 (focal length 36 mm) with a number 1 eyepiece as the 'examining' system, to be very suitable and our measurements have been made on this basis. The focal distance of the lens, which is required in the calculations, is that given as 'equivalent focal distance' in the maker's catalogue.

Provided the above requirements are available, observations on diffraction can be made with no more trouble than that involved in an ordinary microscopic

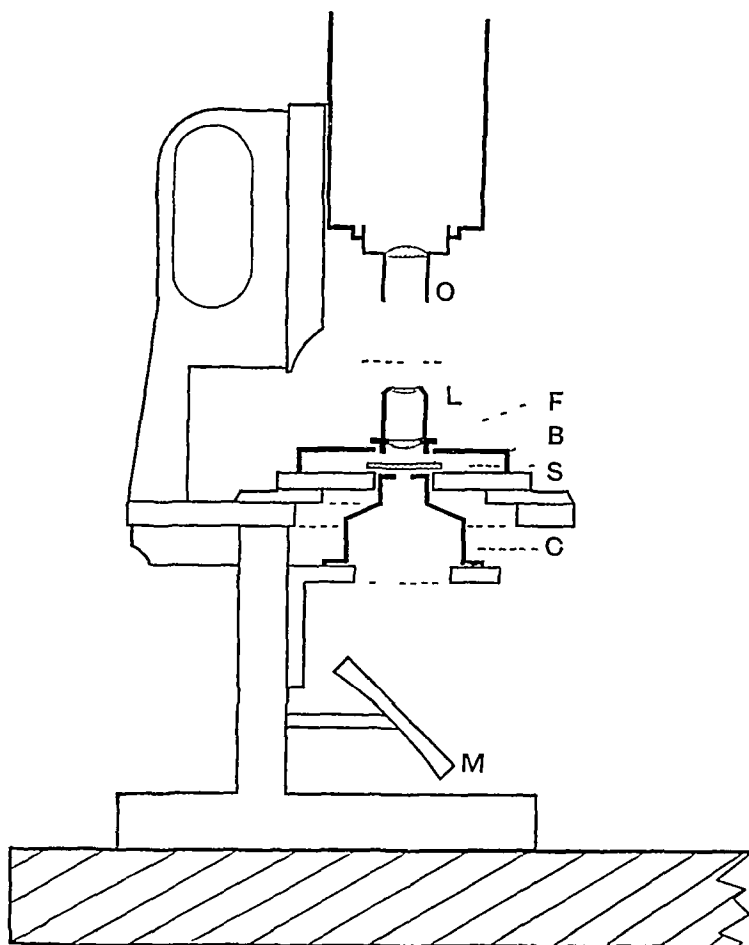


Fig 2

Showing how the microscope is fitted up

- M —Plane mirror by which the parallel rays of light are reflected up through the preparation, S, to be examined
 - C —A cylinder diaphragm of 4 mm aperture under the stage in place of the usual condenser and placed so as almost to touch the slide S
 - B —A movable metal bridge on which the objective L, inverted, is carried by means of the flange F
 - O —The objective of the ordinary microscopic examining apparatus
- The dotted line above L indicates where the diffraction image is brought to a focus by the lens L

examination An advantage in the microscopic method is, that it gives the effect using only a small portion of the film which can be marked, and, if necessary, actual measurement of cells with a micrometer can be made in the same limited field

Appearance of the Diffraction Image.

On examining the thin end of an ordinary well-made blood film by means of this apparatus a diffraction image is seen, which, from the point of view of brilliancy and purity of colour, is equal to that produced by any other form of apparatus tried by us

In the centre of the field is seen the 'central disc' of bright white light surrounded by the coloured rings of the various spectra. Passing from the centre of the field towards the periphery the first colour to be seen is an ill-defined yellow merging into orange and then red. We have accepted Pyppe's view that these rings represent all that can be seen of the spectrum of the 'first order'. In the case of a blood smear containing red cells of 7.8μ average diameter, for example, the maximum radius of the red ring is about 1.2 mm.

Next comes the spectrum of the 'second order' and this is complete. The inner edge of the violet is usually well defined, there being only a slight overlapping with the first spectrum. The outer edge of this ring is not very clear and appears to merge through blue into the green. The next ring is the yellow which is bright and well defined, and measurement of the radius of its *brightest part* is the most convenient to use together with the wave length for yellow light, or $\lambda = 0.59 \mu$. This measurement is referred to hereafter as 'r'. Red cells of 7.8μ , for instance, give 'r' = 1.99 mm. The red or outer ring of the spectrum is separated from the yellow by a thin orange band.

Of the spectrum of the 'third order' only the green and part of the yellow can ordinarily be seen since its outer rings are beyond the field of the microscope and the inner ones are overlapped by the second spectrum, the red of the latter appearing to merge with the green of the third spectrum. As is explained in text-books on physics, the diameter of these rings varies inversely as the diameter of the cells and the brilliancy is adversely affected by irregularity in the shape and size of the cells. Fig. 3 (drawn to scale) is an example of the different sizes of spectra produced by different sizes of red cells. The half spectrum on the left is from human cells averaging 7.79μ in diameter, and that on the right from horse cells averaging 5.61μ in diameter.

The above description refers to the diffraction image produced by a dry film, but the effects given by red cells in a fluid medium such as serum are just the same. For wet preparations we found the ordinary Thoma-Zeiss blood-counting apparatus to be quite suitable. Blood taken from the finger is diluted, by means of the diluting pipette, with previously prepared serum from the same case, about 1 in 100 to 1 in 200 and a small drop placed in the cell of the slide, the preparation being

* Further information as regards the inferences to be drawn from the various appearances of the diffraction image produced by blood smears will be found in Pyppe's article in the *Brit Med Jour*, April 6, 1929, pages 635-637.

covered with an *ordinary* thin coverslip After a minute or two the cells will have settled down and, provided the correct dilution has been made, can be seen lying side by side as in a thin dry film A certain amount of rouleaux formation takes place eventually, but before this happens there is usually ample time for the measurement of the diffraction image In Table II are given figures obtained by this method showing the diameter of red cells suspended in serum as compared with that of cells in dried films prepared at the same time

By the theory of diffraction spectra, when white light passes through a diffraction grating the different colours of which it is composed are diffracted through

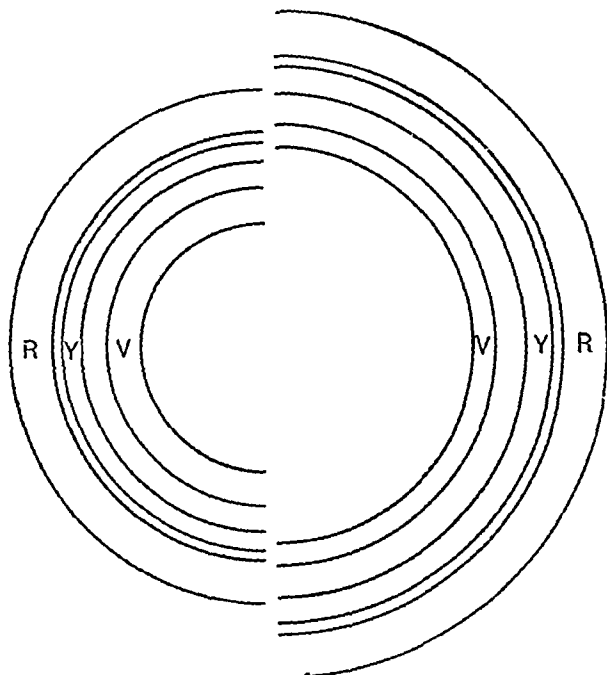


Fig 3

different angles The relation of the width of the bars of the grating to the amount of dispersion of the coloured bands in the spectrum is dealt with in textbooks on Physics and is given by the formula $d = \frac{n \lambda}{\sin \theta}$ where 'd' is the distance between the lines on the grating from centre to centre or, in the case of a blood film as shown later, the diameter of the red cell, θ is the angle of diffraction of a particular colour of wave length ' λ ', and 'n' a constant

By placing a lens between the grating and a screen which is in the focal plane of the lens, each wave length is brought to a focus on this screen thus forming a series of bands of light of different wave lengths If the rulings on the grating are uniform, then it may be assumed that the rays of diffracted light for each wave

length before they enter the lens are parallel and, therefore, that the distance between the lens and the grating is immaterial

Fig 4 explains how the formula is modified by the use of a lens The numbers 1 to 5 represent parallel rays of diffracted light being brought to a focus by a lens, L, at a point F^1 on the screen S, and one of which passes through the optical centre of the lens NW represents a ray of light passing along the optical axis of the lens and striking the screen perpendicularly at W, the centre of the white 'central disc' of the diffraction image OW is the focal distance of the lens,

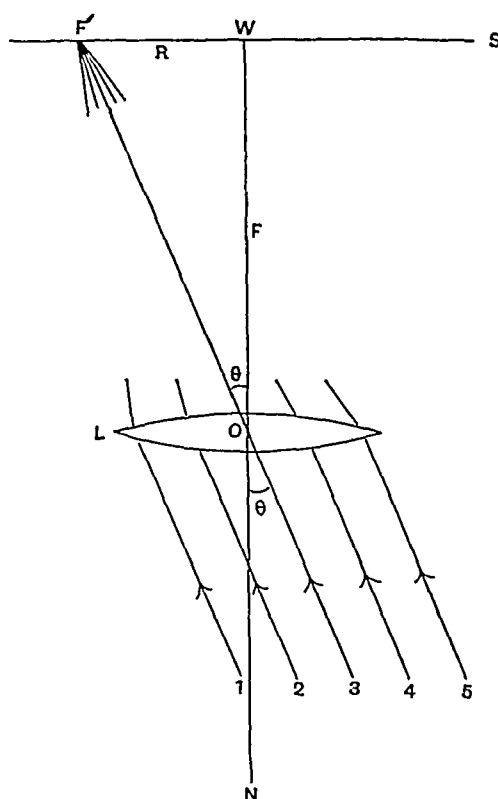


Fig 4

and F^1W the radius of the coloured ring formed by the diffracted rays Therefore, $\sin \theta = \frac{F^1W}{F^1O} = \frac{F^1W}{\sqrt{(OW)^2 + (F^1W)^2}} = \frac{r}{\sqrt{F^2 + r^2}}$ Substituting this value of $\sin \theta$ in the above formula we derive the formula $d = \frac{n \lambda \sqrt{F^2 + r^2}}{r}$ which is that used in calculating the diameter of the red cell from observed '1'

The only value here about which there is any difficulty is 'n' On theoretical grounds, 'n' = 1 where 'r' is measured from one of the bright rings of the first spectrum, or 'n' = 2 in the case of the second spectrum, and 'n' = 3 in the case of the third spectrum and so on—'n' being a whole number With a

diffraction grating we found on actual trial (using white light and measuring the yellow ring) the values of 'n' to be 0.99 for the first spectrum, 1.996 for the second and 2.88 for the third (The accurate measurement of 'r' for the third spectrum was difficult owing to marked loss of brilliancy of this spectrum)

This, however, does not apply to the circular diffraction spectrum produced by red cells, where, from actual trials as against red cells measured with the micrometer, the value of 'n' was found to be 1.7 for what we have taken to be the second spectrum

Several authors on optics refer to the radius of the diffraction rings as being greater in the case of a circular aperture as compared with the edges of a slit Wright (1906) says about $\frac{1}{5}$ greater, in which case 'n' = 1.2 for the first spectrum, 2.4 for the second, and so on We have not, however, been able to find any mention of the exact theoretical basis on which this calculation is made

The cause of the diffraction spectrum as produced by red cells

The diffraction spectrum given by a preparation of red cells is in the form of concentric coloured circles as described in a previous paragraph

The way in which the red cell causes this particular diffraction effect was not at first obvious

Since the red cell is not perfectly opaque to light, some experiments were made with red cells stained black on a clear background as compared with unstained cells on a clear ground If an isolated cell, in a smear which has been stained black, the background between cells being clear, is examined under an ordinary microscope ($\frac{1}{6}$ obj.) without condenser, and illuminated as shown in Fig. 2, and so arranged that the image of the aperture in the screen is exactly in the centre of the cell (this adjustment must be accurate), then the following appearances are seen With the cell *in focus* there are superimposed upon it very faint dark and coloured rings *but no definite central disc*, round the edge of the cell are seen faint coloured bands If the microscope tube is now slowly racked up, a point is reached when the central disc appears and we now have the usual image as formed by an opaque disc placed in the path of a beam of light going towards a screen The reason for the central disc not being visible when the cell is in focus is that, taking any point on the axis of the disc (or red cell), the nearer this point approaches the disc the less its illumination becomes, because the rays of light proceeding from the edge of the disc become more and more oblique and hence the effective interfering rays become more and more cut off (Preston, 1928a) When the point reaches the disc (i.e., when the cell is in focus) no definite central illumination is seen

On the other hand, when an *unstained* cell is examined in the same way, the appearances seen are quite different. With the cell in focus, a bright diffraction image is seen. In the area of the cell is a vivid central disc surrounded by dark rings and coloured rings (2 of each). Outside the cell area are seen two or three coloured bands.

When the microscope tube is racked up slowly the central disc changes from white to black, and vice versa, in rapid succession till a point is reached where it remains white (and larger) and surrounded by coloured concentric rings.

When a very thin wire is placed in the path of a narrow beam of light proceeding to a screen, the image produced on the screen consists of two sets of fringes, one on either side of the image of the wire, and the other *inside* the image of the wire. The diffraction spectrum is caused chiefly by disturbances produced at points corresponding to the diameters of the wire which the light rays just graze (Franklin and McNutt, 1924a, and Preston, 1928b). From these observations it appears quite probable that the unstained red cell does not act precisely as an *opaque* disc but, that the diffraction spectrum is caused both by the outer and inner dark edges of the rim-like outline of the cell (cf a ring of wire), and that the rings must be produced mainly by points on the edges of the rim of the cell, giving interference effects with opposite points of the circumference of the cell, the diameter of which therefore corresponds approximately to the interval between the grating intervals of a diffraction grating as figured by Franklin and McNutt (1924b).

Determination of the Value of 'n'.

After studying the subject at some length, it appeared that the theoretical determination of the value of 'n' in the case of the diffraction produced by red cells was somewhat uncertain.

According to textbooks on optics, the first bright spectrum formed by a diffraction grating appears when the value of the factor 'n' is 1, the second when 'n' is 2 and so on. In the formula given in the earlier of his papers Pijper uses these values, in a later paper (1929) he uses 1.7 as the value of 'n' but gives no indication as to how this figure was arrived at, whether theoretically, or as the result of experimental determination. In determining the value of 'n' experimentally, we used human red cells as well as those of the animals noted in the following table (Table I) where the values of 'r' as determined by the diffraction method and of 'd' as determined by actual micrometer measurements are given. From these the values of 'n' are worked out and shown in column 4. It will be seen that the value of 'n' varies in different cases from 1.64 to 1.72 with an average of 1.69 using dried blood films and 1.7 with wet. We have taken the value of 'n',

therefore, for purposes of calculation as 1.7 This is the same figure as was used by Pijper as noted above

TABLE I

The determination of the value of 'n' from actual measurements
A Using dried blood films, and the apparatus described in text

Source of red blood cells	'r' (in microns)	'd' calculated (in microns, and 'n' = 1.7)	'd' measured (microns)	'n' calculated from observed values of 'r' and 'd'
Normal horse	2764.54	5.75	5.61	1.66
Rabbit	2176.34	7.26	7.32	1.72
White rat	2352.80	6.73	6.62	1.68
Goat	4411.50*	3.68	3.55	1.64
Anemic horse	2646.90	5.99	5.97	1.69
Sheep	2882.18	5.52	5.30	1.65
Guinea-pig	2088.11	7.63	7.70	1.72
A. C. C.	1999.88	7.88	7.79	1.68
L. Singh	1941.06	8.12	8.19	1.71
Valentine	1911.65	8.24	8.28	1.71
Puran	2014.58	7.83	7.72	1.68
			Average	1.69

B Using blood cells diluted in serum and a lower powered lens in place of L (Fig. 2) and measuring 'r' on a screen placed above it

Rabbit	5.25		6.94	1.69
White rat	5.75		6.73	1.79
Sheep	7.25		4.82	1.62
Guinea pig	5.00		7.54	1.72
			Average	1.705

* Owing to the extremely small size of the goat's red cells the yellow band in the diffraction image was projected just beyond the field of view in our apparatus and hence the value of 'r' cannot be taken as strictly accurate.

Comparison of results from 'wet' and 'dry' preparations.

Comparison of the results obtained with human cells diluted in serum as previously described and with the cells measured in dry films is given in Table II. It will be seen that contrary to what might have been expected the amount of difference is very small amounting on the average to about 0.3μ less in the dried than in the wet film.

Some experiments with dried films made from blood diluted with serum so as to reduce the number of cells also gave readings not noticeably different to the usual dried film, though the intensity of the spectrum being reduced thereby readings could not be made so critically.

There appears reason to believe therefore that dried preparations of blood in most circumstances will give results only slightly different to wet preparations and the use of dried films for measuring the size of red cell by the halometric method is therefore justified.

With this result which appears to us important we close the present paper as further work by us dealing with the effects of hypotonic salt solutions and other circumstances affecting the size of the cell, such as surface tension, chemical and immunological effects, appear best dealt with as special contributions.

TABLE II

Measurements, by the diffraction method, of human red cell in dried films and in serum suspensions (= wet preparation)

Number of case	'r' IN MICRONS		'd' (CALCULATED) IN MICRONS	
	Dried film	Wet preparation	Dried film	Wet preparation
1	1852.8	1823.4	8.50	8.64
2	1852.8	1823.4	8.50	8.64
3	1882.2	1823.4	8.37	8.64
4	1852.8	1794.0	8.50	8.77
5	1852.8	1794.0	8.50	8.77
6	1911.6	1823.4	8.25	8.64
7	1882.2	1823.4	8.37	8.64
8	1823.4	1764.6	8.64	8.92
9	1894.0	1823.4	8.32	8.64
10	1894.0	1823.4	8.32	8.64
11	1764.6	1735.2	8.92	9.08

TABLE II—*concl'd*

Number of case	'r' IN MICRONS		'd' (CALCULATED) IN MICRONS	
	Dried film	Wet preparation	Dried film	Wet preparation
12	1852.8	1794.0	8.50	8.77
13	1894.0	1882.2	8.32	8.37
14	1852.8	1794.0	8.50	8.77
15	1852.8	1764.6	8.50	8.92
16	1852.8	1764.6	8.50	8.92
17	1911.6	1823.4	8.25	8.64

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THE SYNTHESIS OF VITAMIN B₁ AND 'BIOS' BY *BACILLUS VULGATUS*

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[Received for publication, October 14, 1931]

INTRODUCTION

THE ability of certain micro-organisms to synthesize 'vitamin B' both in the alimentary tract of some animals and in artificial media has been demonstrated for some years. Heller, McElroy and Garlock (1925) found that certain spore-bearing organisms present in the intestinal tract of rats could synthesize vitamin B. That the rumen of the cow contains a type of *flavo bacterium*, which normally synthesizes 'vitamin B', has been shown by Bechdel *et al* (1928). Calves have been brought to maturity on diets containing very little vitamin B. The phenomenon of refection (Friederica *et al*, 1927, Roscoe, 1927, Kon and Watchorn, 1928), though its nature is still obscure, appears to indicate that under certain conditions organisms in the intestinal tract of rats can synthesize and supply sufficient vitamin B to make the animals entirely independent of an outside source.

The synthesis of 'vitamin B' by *B. coli* in an artificial medium has been effected by Hosoya and Kuroya (1923). Vitamin B₁ and 'bios' can also be similarly produced by certain strains of yeast (Peskett, 1927, Copping, 1929). Scheunert and Schieblisch (1927) have successfully grown *Bacillus vulgaris* (Flügge) in an artificial medium and have found that the dried bacilli can supplement a vitamin B deficient diet for the growth of young rats.

The present work was started in order to investigate whether the process of production of vitamin B₁ in a simple artificial medium containing known constituents would be of greater advantage so far as the isolation of the vitamin was concerned than the usual methods, which start with materials such as yeast and wheat germ, containing many unidentified substances. *Bacillus vulgaris* (Flügge) was chosen for this work and it was sought (1) to estimate the amount of

vitamin B₁, that it is capable of synthesizing in a simple synthetic medium, (2) to investigate if the synthesized vitamin is secreted into the surrounding medium or is kept endocellularly bound, and (3) to find out whether the presence of histidine in the medium would lead to an increased yield of the vitamin. Obviously, if the synthesized vitamin is kept bound inside the cell, this method can offer no substantial advantage over the ordinary methods of fractionation, which start with the yeast cell. The third point was investigated in view of the suggestion of Jansen and Donath (1926) that the vitamin possibly contains an imidazole nucleus. Incidentally, the power of *Bacillus vulgaris* to synthesize 'bios' has been investigated.

EXPERIMENTAL

The culture of *Bacillus vulgaris* (Flügge), used in these experiments, was obtained from the National Collection of Type Cultures. This was subcultured on tryptic broth agar slopes and cultures, which were less than 48 hours old.

The 'synthetic' medium was approximately the same as that used by Scheunert and Schieblieh (1927) and had the following composition —

	(g)
Dextrose	30.0
Malic acid	10.5
Microcosmic salt	15.0
Magnesium sulphate	0.6
Calcium chloride	0.02
Dipotassium hydrogen phosphate	3.0
Ferrous sulphate	trace

These substances were all of the purest variety obtainable (Kahlbaum or B. D. H. 'A. R.' products). The malic acid was separately weighed out and neutralized with the requisite amount of *N* NaOH. All the constituents were dissolved in distilled water, brought to pH 7.0 (electrometrically) and made up to 1,550 c.c. The vitamin B₁ content of this medium was estimated (*see later*) and was found to be nil. The medium was sterilized in Roux bottles by heating for 20 minutes on three successive days in a steamer. In order to have as little preformed vitamin B₁ and 'bios' as possible in the inoculating bacterial suspension the following procedure was adopted. A fresh culture of *Bacillus vulgaris* on an agar slope was gently stirred with 5 c.c. of sterilized Ringer's solution and 0.2 c.c. of this was inoculated into 150 c.c. of the sterile medium in one Roux bottle, which was then incubated at 37°C for 72 hours. 0.2 c.c. of the bacterial suspension in this Roux bottle was then inoculated into the sterile medium (150 c.c.) in each of the other Roux bottles, which were subsequently incubated at 37°C for 6 days. The medium of the first Roux bottle, containing the bacterial suspension with which these

inoculations were made, was discarded. There were signs of growth during the first 24 hours, and the growth was quite vigorous during the next 24 hours. The rate of growth fell off after the first 72 hours. The bacilli always tended to accumulate as a scum on the surface. The pH of the medium (7.0) changed to 6.50 after 3 days' incubation and to 8.67 after 6 days' incubation. The fall in pH followed by a rise is apparently due to the production of organic acids, which are subsequently combusted, leading to an accumulation of alkaline carbonates.

The vitamin B₁ potency of the dried bacilli

After 6 days' incubation, the bacilli, which had grown in 1,350 c.c. of the medium, were centrifuged off. The residue of bacilli was dried first at 50° and then in a vacuum desiccator (dry weight = 1.3051 g). By the same method other batches of *Bacillus vulgaris* were grown and the yield was of the same order.

Each lot of 1.3 g. of the dried organism was boiled up with 15 c.c. distilled water and 0.1 c.c. glacial acetic acid for 1½ minutes and filtered under suction. The slightly opalescent solution was fed to polyneuritic pigeons and the average day-dose was computed by the usual method of dividing the dose administered by the number of days for which protection was afforded. The general technique for assay has been described elsewhere (Guha and Drummond, 1929).

Dose administered, expressed as equivalents of the dried bacilli (g)	Number of days of protection	Day dose (g)
1.3	4	0.325
1.3	5	0.26
1.3	4	0.325
1.3	4	0.325
1.3	3.5	0.37
1.3	13	..

* This test was neglected as probably an abnormal case.

Hence the average day-dose is equivalent to 0.321 g. of the dried bacilli.

The vitamin B₁ potency of the medium after separation from the organisms

The centrifugate from the bacilli was somewhat opalescent, and in order to remove the last traces of the organisms, this was rapidly filtered through a very thin layer of kieselguhr under suction. As the pH of the medium was 8.67 the

vitamin was not appreciably absorbed by the kieselguhr (Guha, 1931) The filtrate was then faintly acidified with hydrochloric acid, and, as it contained a fair amount of inorganic material, the vitamin in it had to be somewhat concentrated before feeding The following operation was repeated for different batches of the medium

One thousand two hundred c c of the medium were concentrated under reduced pressure to 275 c c and treated with 275 c c of absolute alcohol After standing overnight in the refrigerator the crystalline precipitate was filtered off and washed with 70 c c 50 per cent alcohol The precipitate was almost entirely inorganic The filtrate was concentrated under reduced pressure to 165 c c, filtered from a further quantity of precipitated inorganic material and treated with concentrated H₂SO₄ to make it 5 per cent acid and then with 80 c c of saturated phosphotungstic acid in 5 per cent H₂SO₄ After standing overnight the phosphotungstic precipitate was filtered off, sucked dry on the Buchner funnel (weight = 20 g), ground up with 200 c c of 50 per cent acetone, filtered from a small quantity of undissolved residue and reprecipitated with 750 c c of 5 per cent H₂SO₄ After standing for 24 hours the precipitate was removed, washed with 5 per cent H₂SO₄ and then with a small quantity of water, dissolved in 150 c c of 50 per cent acetone and decomposed by saturated baryta, the excess barium being removed from the filtrate by sulphuric acid The solution was concentrated under reduced pressure before administration

Several batches of the medium were worked up in the above way after removing the organisms Tests on pigeons, however, yielded practically negative results in doses corresponding to nearly 800 c c of the medium

The vitamin B₁ potency of the medium before inoculation with *Bacillus vulgatus* was tested after a similar fractionation with entirely negative results

*The 'bios' activity of the medium before and after growing
Bacillus vulgatus **

The following basal medium was used for the growth of *Saccharomyces cerevisiae* —

	Per cent
Sucrose	2
Ammonium chloride	0 25
Magnesium sulphate	0 25
Potassium chloride	0 25
Disodium hydrogen phosphate	0 25
Calcium carbonate	0 05

* I am indebted to Dr C Elvehjem for his help in this part of the work.

TABLE

This medium was found to contain 0.2 mg. per 100 c.c.

The results of the 'bios' tests are given in the following table —

Volume of basal medium used for the growth of yeast (c.c.)	Nature of addendum	Volume of addendum (c.c.)	Dry weight of the inoculum of yeast cells (mg.)	Dry weight of yeast cells after 48 hours' incubation at 25° with continuous aeration (mg.)
50			5	166
50	<i>B. vulgaris</i> medium before inoculation with <i>B. vulgaris</i>	4	5	220
50	<i>B. vulgaris</i> medium filtered after 72 hours' growth of <i>B. vulgaris</i>	2	5	252
50	<i>B. vulgaris</i> medium filtered after 6 days' growth of <i>B. vulgaris</i>	2	5	329

Unfortunately, in the above experiments the size of the inoculum of yeast cells happened to be somewhat too large, which would tend partially to mask the 'bios' effect of the addendum. Even so, the figures quoted show that 2 c.c. of the *Bacillus vulgaris* medium, obtained after the bacilli have grown for 6 days in it, have a relatively much greater stimulating effect on the growth of yeast than 4 c.c. of the fresh *Bacillus vulgaris* medium. It may be noted that the 'bios' effect of the medium after 3 days' growth of *Bacillus vulgaris* is relatively slight. This might either mean that the production of 'bios' is comparatively slow during the first 3 days or that the 'bios' synthesized is liberated to some extent during the last 3 days of incubation due to partial autolysis of the cells. Assuming the minimum detectable dose of 'bios' to be lower than the minimum detectable dose of the vitamin, it is possible that such partial autolysis, while indicating the presence of 'bios', would not reveal the presence of the vitamin in the medium.

*The effect of histidine on the production of vitamin B₁ by
Bacillus vulgaris*

In the medium described before, 0.75 g. malic acid was replaced by the same amount of histidine, and the procedure for inoculation and incubation was the same.

The dried bacilli corresponding to 1,350 c c of the medium weighed 1.2012 g. The weights of the organisms obtained in different batches of the medium were approximately of the same order. There is, thus, no stimulating effect of histidine on the growth of *Bacillus vulgaris* in this medium.

Pigeon-curative tests with the aqueous extracts of these batches of organisms gave the following results —

Dose fed, expressed as equivalents of the dried bacilli (g.)	Number of days of protection	Day-dose (g.)
1.2	4	0.3
1.3	3	0.43
1.2	3	0.4
1.4	3	0.47

Hence the average day-dose corresponds to 0.40 g. of the dried bacilli. A single dose, corresponding to 0.6 g. of the dried bacilli, failed to effect a cure.

The medium, after removal of the bacilli, was concentrated in the manner described before and was found to be inactive.

Thus histidine had no perceptible influence on the synthesis of the vitamin by *Bacillus vulgaris*.

SUMMARY

(1) *Bacillus vulgaris* is not able to synthesize sufficient vitamin B₁ in a simple synthetic medium to make the process a convenient one for the concentration of the vitamin.

(2) As the vitamin so synthesized is not secreted into the medium, this process does not offer any advantage over ordinary methods of fractionation, which start with materials like yeast.

(3) *Bacillus vulgaris* is able to synthesize 'bios' in a simple synthetic medium.

(4) The presence of histidine in the medium has no effect on the synthesis of the vitamin by *Bacillus vulgaris*.

ACKNOWLEDGMENTS

I wish to express my gratitude to Professor Sir F. G. Hopkins for his kind interest in this work.

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[The following has been received from the War Office.—Ed.]

LONDON

21st September, 1931

NORTH PERSIAN FORCES MEMORIAL MEDAL.

DR T F ANDERSON, Colonial Medical Services, has been awarded the North Persian Forces Memorial Medal for the year 1930 for his paper on a ' Report on an Investigation of Health Conditions on Farms in the Trans-Nzozia, with special reference to Malaria ', published in the *Kenya and East African Medical Journal*, Vol VI, No 10, January, 1930, pp 274-306

The North Persian Forces Memorial Medal is awarded annually for the best paper on Tropical Medicine or Hygiene published in any Journal during the preceding twelve months by a Medical Officer, of under twelve years' service, of the Royal Navy, Royal Army Medical Corps, Royal Air Force, Indian Medical Service, or of the Colonial Medical Service, provided the Memorial Committee consider that any of the papers published has attained a standard of merit justifying an award

THE COMPARATIVE VALUE OF ANTI-PLAGUE BILIVACCINE AND HAFKINE'S PLAGUE PROPHYLACTIC.

CORRIGENDA

- (1) In paper entitled 'The Effect of Intravenous Injections of Quinine on the Electrocardiogram in Man' by Lt Col T A Hughes, I M S, in the July 1931 (Vol XIX, No 1) number of the *Indian Journal of Medical Research*, on page 119, line 7 of CONCLUSIONS read 'occurred' in place of 'occupied'
- (2) In paper entitled 'Leucocyte Changes following Injection of Sanocrysin in Pulmonary Tuberculosis and their Significance' by Lt Col T A Hughes, I M S, and Dr D L Shrivastava, in the October 1931 (Vol XIX, No 2) number of the *Journal*, on page 585, line 8, read 'monocyte lymphocyte ratio' in place of 'monocyte leucocyte ratio'
- (3) In paper entitled 'Studies in the Nutritive Value of Indian Vegetable Food-Stuffs, Part III', by Dr S P Niyogi, M B, M Sc, Messrs N Narayana, M Sc, and B G Desai, B Sc, in the January 1932 (Vol XIX, No 3) number of the *Journal*, on page 860, Table I, against '*Lens esculenta*' read as follows in place of the figures given —

Pulse	Ash	Ether ex- tractives	Crude fibre	Crude protein N \times 6.25	Carbo- hydrates (by differ- ence)	True pro- tein (deter- mined sep- arately)
<i>Lens esculenta</i>	2.28	1.10	1.22	28.44	66.96	24.21

Anti-plague bilivaccine

Messrs G Loucatos and Company, Bombay, the agents of La Biotherapie, 131, Rue Cambonne, Paris (XVe), addressed letters to the Director, Haffkine Institute, Bombay, to the Director, Central Research Institute, Kasauli, and to the Secretary, Indian Research Fund Association, with a request to test the efficacy of anti-plague bilivaccine prepared according to a formula based on the researches

THE COMPARATIVE VALUE OF ANTI-PLAGUE BILIVACCINE AND HAFKINE'S PLAGUE PROPHYLACTIC.

BY

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AND

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[Received for publication, October 16, 1931].

BACTERIAL vaccines have largely been employed with benefit as a measure of protection against infectious diseases such as cholera, plague and typhoid. It is generally believed that their use would be still more popular if it were not for the local and general reactions which usually follow their injections. Attempts have been made from time to time to prepare detoxicated vaccines without impairing their potency. The results so far, however, have not been satisfactory.

Professor Besredka, the originator of the local immunity theory, introduced the method of administering vaccines by the mouth. For this purpose, bile is given on an empty stomach followed by the vaccine one half hour later. Both the bile and the vaccine are administered in the form of pills. Anti-cholera, anti-typhoid and anti-dysentery bilivaccines are now commercially available for prophylactic use. The advantages claimed for the employment of bilivaccines are (1) the bile and the vaccine pills are easily swallowed, (2) they are free from any after effects, (3) their administration does not necessitate the presence of a medical attendant, and (4) being dry, they keep indefinitely.

Anti-plague bilivaccine

Messrs G Loucatos and Company, Bombay, the agents of La Biotherapie, 131, Rue Cambonne, Paris (XVe), addressed letters to the Director, Haffkine Institute, Bombay, to the Director, Central Research Institute, Kasauli, and to the Secretary, Indian Research Fund Association, with a request to test the efficacy of anti-plague bilivaccine prepared according to a formula based on the researches

of Besiedka and containing per tablet 50 milligrams of heat-killed and desiccated plague bacilli which represent from 60 to 70 billion organisms. Their object was to determine the comparative protective value of anti-plague bilvaccine administered orally with that of anti-plague inoculation. This paper deals with our experiments on the immunizing value of anti-plague bilvaccine as compared with that of Haffkine's plague prophylactic in laboratory animals.

Experimental animals

Rats, guinea-pigs and rabbits are the principal laboratory animals which are highly susceptible to experimental infection with plague. Morison, Naidu and Avari (1924) found that rats were not suitable animals for the oral administration of bile. They could not be induced to eat baits prepared with bile and when bile was administered to them by a catheter they speedily died apparently of oedema of the glottis. Guinea-pigs, however, could be fed with bile by a catheter. The treated guinea-pigs which were given doses similar to those given by M. Legar and A. Baury (1923) afforded no evidence that any immunity was conferred by the oral administration of bile and Haffkine's plague prophylactic or by the oral administration of the plague prophylactic alone.

In our previous experiments on the treatment of plague, we found that the rabbits were not only highly susceptible to infection with plague but they also yielded results which were consistent and regular when the experiments were repeated several times. In view of this experience, we employed rabbits as the test animals in our present experiments.

Administration of vaccines

For the immunization of rabbits, the vaccines were employed in doses recommended for man. In the case of anti-plague bilvaccine we followed the directions given by the manufacturers, namely (1) to give on an empty stomach one pill of bile followed one half hour later by one tablet of vaccine, (2) to give feed to animals only an hour after ingestion of the vaccine, and (3) to repeat the treatment on two successive days. Rabbits selected weighed from 1,310 to 2,440 grammes and they were given their last feed in the evening and immunization with anti-plague bilvaccine pills was commenced the next morning on an empty stomach. In the first instance, we found difficulties in making the rabbits swallow these pills because of their size, the pills were, therefore, administered in liquid form by dissolving the bile pill in slightly alkaline water and the vaccine tablet in normal saline. With some practice we were able later on to introduce the pills entire by pushing them back down the throat into the oesophagus. The pills were administered on three consecutive mornings. In the case of Haffkine's plague prophylactic we employed a vaccine which had been stored in the laboratory for a little over six months.

after its manufacture and injected it subcutaneously in a dose of 4 c c as recommended for man

Test dose of plague

The test virus employed was the spleen of a rat that had died of acute plague. A portion of this spleen was weighed and then ground in a mortar with sterile sand. It was then emulsified with a sufficient quantity of broth so that each cubic centimetre of the emulsion contained 0.003 milligram of plague spleen by weight. One cubic centimetre of this spleen emulsion was the test dose employed for infection. With this amount injected subcutaneously into rats, guinea-pigs and rabbits there is invariably a mortality of 80 to 100 per cent among these animals within 10 days after infection.

Estimation of protective value of the vaccines

In the first two series of experiments we allowed an interval of 14 days between the immunization with vaccines and the infection with the test dose, in the third series this interval was reduced to 7 days. The protective value of the vaccines was estimated by the number of survivors after a period of 30 days following the infection. Every rabbit that died was examined post-mortem, smears were made from the bubo, spleen, liver, lungs and heart blood and examined microscopically, and further animal passage was made by rubbing the spleen of the rabbit on the shaved surface of the abdomen of a rat in order to confirm that the death had occurred from plague.

Results of the experiments

We have summarized the results of these experiments in the following Tables I, II and III.

Summary

The anti-plague bilivaccine pills when administered orally to rabbits, either dissolved or entire, did not confer any immunity against a test dose of plague injected after an interval of 7 or 14 days later. There was, in fact, no difference in the percentage mortality between the animals thus immunized and the untreated controls.

Under identical conditions of experiment, however, when Haffkine's plague prophylactic was administered by subcutaneous inoculation in a dose of 4 c c it gave 100.0 per cent protection.

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The immunizing value of anti-plague bubovaccine and Haffknecht's plague prophylactic in rabbits.

(Bilvaccine pills were dissolved and administered in liquid form, the interval between immunization and infection was 14 days)

Survivors after 30 days of infection													Percentage of survivors = 100
Unprotected controls Daily mortality after infection													
1	2	3	4	5	6	7	8	9	10	11			
					D						D		
					D								
					D								
Weight in grammes													
												1,300	
												1,300	
												1,320	
												1,360	
												1,370	
												1,380	
												1,390	
												1,390	
												1,400	
												1,400	

Survivors after 30 days of infection													Percentage of survivors = 100
Protected with Haffkine's prophylactic Daily mortality after infection													
1	2	3	4	5	6	7							
Weight in grammes													
												1,460	
												1,520	
												1,420	
												1,520	
												1,630	
												1,400	
												1,510	
												1,470	
												1,400	
												1,480	

Survivors after 30 days of infection													Percentage of survivors = 100
Protected with anti plague bilvaccine Daily mortality after infection													
1	2	3	4	5	6	7							
Weight in grammes													
												1,560	
												1,420	
												1,500	
												1,550	
												1,400	
												1,800	
												1,480	
												1,430	
												1,540	
												1,410	

TABLE II

The immunizing value of anti-plague bilvaccine and Haffkine's plague prophylactic in rabbits

Bilvaccine pills were administered entire, the interval between immunization and infection was 14 days

Weight in grammes	Protected with anti plague bilvaccine Daily mortality after infection								Survivors after 30 days of infection	Weight in grammes	Protected with Haffkine's prophylactic Daily mortality after infection								Survivors after 30 days of infection	Weight in grammes	Unprotected controls Daily mortality after infection								Survivors after 30 days of infection	
	1	2	3	4	5	6	7	8			1	2	3	4	5	6	7	8			1	2	3	4	5	6	7	8		
1,820								D		1,920									1	1,470										
1,870								D		2,170									1	1,510										
1,910										2,240									1	1,520										
1,940										1,650									1	1,510										
1,970										1,670									1	1,510										
2,020										1,700									1	1,590										
2,040										2,250									1	1,650										
2,220										2,180									1	1,660										
2,250										2,170									1	1,730										
2,440										2,150									1	1,750										
Percentage of survivors = 0 0										Percentage of survivors = 100 0										Percentage of survivors = 0 0										

* Note—Omitted as it gave birth to young ones before infection

STUDIES ON SERUM PROTEINS IN LEPROSY, WITH SPECIAL REFERENCE TO HYDROCORTISONE TREATMENT

BY

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[Received for publication, October 21, 1931]

SERUM proteins in leprosy have been studied by Schobl and Basaca (1921) who have reported positive globulin precipitation in leprosy, 'in all forms', and this they attribute to 'an upset balance between salts and globulin—possibly euglobulin'. Frazier and Wu (1925) have reported excess of serum globulin in 17 out of 32 cases of leprosy studied by them, 'of degrees, varying from one to three plus', most marked in nodular cases. Wade (1925), from his studies in the Philippines, has found that the globulin value of the serum increases progressively with the course of the disease tailing off in recovering cases, and has also noted very high values in the phase of 'reaction'. He has also observed a parallelism between formalin coagulation and globulin value, using the original technique of the old formol-gel test. Napier (1927), who has modified the reading of the results in the old formol-gel test so that it may be applicable in kala-azar, denies that the test is positive in uncomplicated cases of leprosy, if his standards of positivity are strictly adhered to. Rogers (1927) while abstracting the paper of Dr Wade, above referred to, in the *Tropical Diseases Bulletin*, remarks that the effect of the Hydnocarpus derivatives on the globulin content, has not yet been ascertained. He further remarks that the formalin coagulation reaction (presumably the old formol-gel test) corresponds in a general way with the globulin values and is almost always positive in cases with significant increase in globulin. He also opines that the formalin coagulation test being much simpler than the very difficult laboratory

globulin value estimations, and being almost parallel to the latter, may therefore prove of great clinical value in detecting incipient cases, before they reach the stage of clinical symptoms

Such hopeful opinions concerning the globulin value estimations and the formalin coagulation test, expressed by Rogers, naturally tempted the writer to undertake a fresh study of the serum proteins in leprosy. Also, the fact that, with the possible exception of Henderson (1927), who has studied this question with special reference to potassium iodide treatment in leprosy, no other worker in India seems to have so far studied this question in Indians with special reference to treatment with *Hydnocarpus* preparations, lent additional support to this investigation

Material and technique

The sera from 252 cases of leprosy, of all types and stages, and of both sexes, all of them inmates of the Purulia Leper Colony, have been studied. As a control, sera from 56 healthy inmates of the 'healthy homes' of the Colony, have been examined. As far as possible, the age groups of the patients investigated and those of the healthy controls have been kept approximately the same. As a further control, however, a few untreated cases of all sub-types of leprosy have also been studied.

Ten c.c. of blood was withdrawn from each case the previous evening and the blood allowed to clot. Next morning, the clear serum was separated and used for the different tests. Haemolysed specimens were discarded and slightly red tinged ones were centrifuged until clear serum separated. Only clear serum has been used for all the tests.

Serum albumin contained in 1 c.c. of serum was precipitated by Spiegler's reagent, which is considered to be an extremely delicate reagent for revealing the presence of even minute traces of albumin. After the albumin was precipitated, the tubes were centrifuged at high speed for 10 minutes, so as to precipitate completely any quantity of albumin that might have been held in suspension in the supernatant fluid. When complete precipitation was thus secured, the clear supernatant fluid was thrown away and the precipitate with the tube weighed accurately in a chemical balance. Subtracting the weight of the tube (ascertained previously) the actual quantity of albumin was calculated correct to two places of decimals.

To save space, the range of albumin values in each group (comprising cases of almost the same type) of cases of leprosy, is given in grammes per c.c. of serum in Table I. The new international nomenclature adopted recently at the Leonard Wood Memorial Conference held at Manila has been used in designating the types and sub-types of cases of leprosy. But, as Dr E. Muir's classification has been in

use for a long time, and as the new international nomenclature has not yet come into general use, the older classification is also given, within brackets —

TABLE I

Types and sub types of cases	Number of cases	SEX		Albumin range in g per cc	Mean albumin value	Age groups of cases in years
		Male	Female			
N ₂ (A ₁ and A ₁ -A ₂)	43	25	18	0.46—0.86	0.66	8—42
N ₃ (A ₂)	32	30	2	0.59—0.87	0.73	12—65
C ₁ (B ₁ and B ₁ -A ₂)	66	18	48	0.59—0.90	0.75	10—60
C ₂ (B ₂ and B ₂ -A ₂)	75	43	32	0.62—1.01	0.82	8—70
C ₃ (B ₃ and B ₃ -A ₂)	36	22	14	0.47—1.00	0.71	12—63
Healthy controls	56	22	34	0.64—1.11	0.88	8—55

A perusal of the figures in this table shows that the albumin values in all types and sub-types of lepers show a significant reduction, as compared with the values for the healthy controls. While the minimum value varies from 0.46 in N₂ cases to 0.62 in C₂ cases, that in healthy controls stands at 0.64. Similarly while the maximum value varies from 0.86 in N₂ cases to 1.01 in C₂ cases, that for the healthy controls stands at 1.11. Both the minimum and the maximum values therefore show a significant decrease from the normal range in the healthy controls.

Next, comparing, the albumin values of the different types and sub-types of cases, C₂ cases, evidently show a relatively higher range of values than all the other sub-types. C₁ and C₃ cases come next. N₂ cases show the least values, both in respect of the minimum and the maximum limits.

Now the question is, whether this significant reduction in the albumin values in lepers of all types, as compared with the healthy controls, is due to leprosy 'per se' or due to any other accompanying condition? To answer this question satisfactorily, as suggested by Dr E. Muir, the erythrocyte sedimentation test has been carried out in all the cases studied including the healthy controls.

It is now well known that albumin, being a bad conductor of electricity, tends to maintain the suspension stability of the erythrocytes, whenever it is in excess, and conversely the suspension stability is lowered, or in other words the sedimentation of the erythrocytes is quickened, whenever there is a deficiency of albumin. Globulin being a good conductor of electricity increases the sedimentation rate whenever it is in excess. Thus, the relative proportion of these two constituent proteins of the blood determines largely the suspension stability of the erythrocytes.

Therefore, the erythrocyte sedimentation test serves to give an idea of the relative proportion in which the albumin and globulin exist, in a specimen of blood, and, as such, is confirmatory to the albumin and globulin value estimations

An analysis of the sedimentation indices of all the cases studied, and of the healthy controls, shows that lepers have relatively higher indices (specially the cutaneous type of cases) even in the non-reacting state, as compared with the healthy controls. It is also noteworthy that the neural type of cases approximate to the healthy standard in the matter of their sedimentation indices, except during the phase of reaction. Even untreated leper controls (mostly cutaneous cases of the 2nd and 3rd degrees) have relatively higher indices than the healthy controls. Therefore, this comparatively higher sedimentation index in cases of leprosy cannot reasonably be attributed to any other cause but leprosy.

Since it has been shown that a high sedimentation rate is caused, among other factors, by a deficiency of albumin *vis-a-vis* an excess of globulin, it is permissible to conclude that leprosy may, by itself, cause a reduction in the albumin value.

An analysis of the sedimentation indices is given below in Table I (a) —

TABLE I (a)

Types of cases	Number of cases	Minimum range	Minimum average	Maximum range	Maximum average
N ₂ (A ₁ and A ₁ A ₂)	43	7—12.0	9.5	24.5—70.0	47.25
N ₃ (A ₂)	32	10—12.5	11.25	46—66.5	56.25
C ₁ (B ₁ and B ₁ -B ₂)	66	7—43.5	25.25	61—74.5	67.75
C ₂ (B ₂ and B ₂ -A ₂)	75	6.5—56.0	31.25	58—78	68.0
C ₃ (B ₃ and B ₃ -A ₂)	36	28.5—45.5	37.0	62—95.5	73.75
Untreated leper controls mostly C ₂ and C ₃ cases	12	34—47	40.5	58.5—79.0	68.75
Healthy controls	56	3.5—20.5	12.0	21—52	36.5

N.B.—(i) The well known technique of Dr. E. Muir has been used and the readings, taken at room temperature, are in hundredths of a c.c.

(ii) The minimum range has been obtained by grouping together the indices of batches of cases of the same type tested on different days.

(iii) The maximum range is obtained similarly, but from different batches of cases, and this group includes a few reacting cases also, in each type.

(iv) The cause of the fairly high indices shown in the maximum range for the healthy controls could not be ascertained by clinical and other simple laboratory examinations.

Serum globulin contained in 1 c.c. of clear serum was precipitated by half saturated ammonium sulphate solution in distilled water. After the precipitation

was apparently complete, the tubes were centrifuged at high speed for 15 minutes, and the clear supernatant fluid was thrown away. The amount of globulin was estimated in exactly the same way as albumin was estimated and the results are given in Table II in grammes per c c of the serum —

TABLE II

Types and sub types	Number of cases	Sex		Globulin range in g per c c	Mean globulin values	Ages of cases in years
		Male	Female			
N ₂ (A ₁ and A ₂ -A ₁)	43	25	18	0.11—0.72	0.42	8—48
N ₃ (A ₂)	32	30	2	0.08—0.70	0.44	12—65
C ₁ (B ₁ and B ₁ -A ₁)	66	27	39	0.07—0.95	0.51	10—60
C ₂ (B ₂ and B ₂ -A ₂)	75	43	32	0.08—0.90	0.49	8—70
C ₃ (B ₃ and B ₃ -A ₂)	36	22	14	0.08—0.88	0.48	12—63
Healthy controls	56	22	34	0.16—0.44	0.30	8—55

N.B. —(1) All calculations correct to two places of decimals

(2) The figures representing the minimum and the maximum globulin values given in this table are not obtained from a single, possibly atypical case, but are the 'average' of a group of cases, in each type

A study of the figures in this table shows that there is a consistently high maximum globulin value in all the types of lepers, as compared with the healthy controls. Thus, while the maximum globulin value ranges from 0.72 in N₂ cases to 0.95 in C₁ cases, that for the healthy controls stands at 0.44. Next comparing the minimum globulin values in the different types of lepers, with that for the healthy controls, it becomes obvious that the minimum globulin values, in some cases, from each type are just half of, or slightly more than half of, the value in healthy controls. This shows that there is a phase, in some of these cases at least, in each type, at which the serum globulin value reaches a subnormal level. This point will be more fully dealt with, when the influence of treatment is considered.

Finally, comparing the mean globulin values in each group of lepers and in the healthy controls, given in column 6, it becomes evident that lepers of all types, except at certain phases, which will be discussed later, have a considerably higher globulin value than the healthy controls. This finding partially confirms those of Schobl and Basaca (1921) and Frazier and Wu (1925).

The effect of treatment with Hydnocarpus products on the serum globulin in lepers

To estimate accurately the influence of Hydnocarpus treatment on the globulin content of lepers' sera it is convenient to divide the cases studied according to the time that has elapsed since an injection was given (by any method) of any of the Hydnocarpus products Table II (a), given below, shows the number of cases studied, with their respective types, ages, etc., and their globulin values, on the 2nd day after an injection of any Hydnocarpus preparation —

TABLE II (a)

Types and sub types	Number of cases	Sex	Ages of cases in years	Phase	Globulin range in g per c c	Mean globulin value	Preparation and dose	Number of cases
N ₂ (A ₁ and A ₁ -A ₂)	2	F	50—55	NR	0.61—0.63	0.62	Hydnocreol 5 c c	All
C ₁ (B ₁ and B ₁ -A ₂)	5	F	24—56	NR	0.61—0.91	0.76	Hydnocreol 5 to 8 c c Ethyl esters 1 c c	4 1
C ₂ (B ₂ and B ₂ -A ₂)	3	F	37—48	NR	0.51—0.77	0.64	Hydnocreol 5 to 8 c c	All
C ₃ (B ₃ and B ₃ -A ₂)	1	F	42	NR	0.65	0.65	Hydnocreol 6 c c	All
Untreated controls C (B) types	12	M ₇ F ₅	20—55	NR	0.11—0.77	0.44	Nil	Nil
Untreated controls N (A) types	30	M	14—65	NR	0.08—0.79	0.14	Nil	Nil

N B —(i) All calculations correct to two places of decimals

(ii) M=Males F=Females NR=Non reacting C=Cutaneous N=Neural

A comparative study of the mean globulin values in column 7 shows that there is a high value in treated cases of all types, as compared with the untreated controls. Also, the minimum globulin values in all the treated cases are considerably higher than the corresponding values in the untreated controls.

The effect of the Hydnocarpus injection seems to be to cause a marked increase in the serum globulin content, in all types of cases, which can be detected on the 2nd day after the injection.

Particulars of cases tested on the 3rd day after an injection are given below in Table II(b).—

TABLE II (b)

Types and sub types	Number of cases	Ages of cases in years	SEX		Phase	Globulin range in g per c c	Mean globulin value	Preparation and dose	Number of cases
			Male	Female					
N ₂ (A ₁ and A ₁ -A ₂)	10	14—70	3	7	NR	0.44—0.67	0.56	Ethyl esters $\frac{1}{2}$ to 4 c c	All
N ₂ (A ₂)	11	12—36	10	1	NR	0.46—0.71	0.59	Hydnocrool 7 to 10 c c	5
C ₁ (B ₁ and B ₁ -A ₂)	13	10—60	6	7	NR	0.07—0.58	0.33	Esters 3 to 5 c c	6
C ₂ (B ₂ and B ₂ -A ₂)	17	12—62	9	8	NR	0.10—0.52	0.31	Esters 1 to 5 c c	7
C ₃ (B ₃ and B ₃ -A ₂)	9	12—63	6	3	NR	0.10—0.54	0.32	Hydnocrool 4 to 6 c c	6
Untreated controls N (A) types	30	14—65	30		NR	0.08—0.79	0.44	Hydnocrool 4 to 10 c c	10
Untreated controls C (B) types	12	20—55	7	5	NR	0.11—0.77	0.44	Esters 1 to 5 c c	7
								Hydnocrool 3 to 10 c c	8
								Esters 3 c c	1

N B —Ethyl esters mentioned above are from *Hydnocarpus wightiana*

A perusal of the figures in this table shows that the minimum globulin values in N types of cases are considerably higher than the corresponding values in C types of cases. The maximum globulin values also, in N types of cases, are considerably higher than in C types of cases. Thus, while the maximum value in N cases ranges from 0.67 to 0.71, that in C cases varies from 0.52 to 0.58 and even here there is a significant reduction of the globulin value in C types of cases. Next, comparing the mean globulin values in N and C types of cases, it is evident that once again C cases show the same significant reduction.

This difference in the globulin values of the two main types of treated cases is not manifest in the untreated leper controls. In fact the globulin values (mean values) of both N and C types of untreated controls are the same. Therefore it is reasonable to conclude that this notable difference in the globulin values of the two main types of lepers (treated cases) must be due to the influence of treatment.

From the table, it is further evident that 16 cases of the N type have been treated with ethyl esters of the *Hydnocarpus wightiana* oil, their doses ranging from $\frac{1}{2}$ to 5 c c. Similarly, 15 cases of the C type have been treated with the same ethyl esters, their doses ranging from 1 to 5 c c. There is thus practically no

difference in either the preparation used or in the dosage. But there is a significant difference in the globulin values of the two main types of cases.

Wade (1925) has reported that the globulin value increases progressively with the duration of the disease, but he has studied the globulin value in cutaneous (C) types of cases alone, and has not ascertained how treatment with *Hydnocarpus* preparations influences the globulin value whether the effect is identical in both the main types of cases or whether it differs. But, from the results of this present study, it is clear that the effect of *Hydnocarpus* treatment is to cause an increase in the globulin contents in all cases of leprosy on the 2nd day after an injection, and thereafter to cause a reduction from the 3rd day onwards (provided no reaction sets in), more rapidly in cutaneous cases than in neural cases.

To follow up further the quantitative changes in the serum globulin content cases have been tested on the 4th, 5th, 6th, 7th, 8th, 9th, 11th and 12th day after a single injection and also from 13 days to 1 month and upwards of a month after a single injection, and the details of such cases are given in Table II (c) —

TABLE II (c)

Types and sub types	Number of cases	Sex		Ages in years	Phisic	Globulin range	Mean value	Preparation and doses	Number of cases	Days after injection
		Male	Female							
N ₂ (A ₁ and A ₁ -A ₂)	3		3	7-15	NR	0.36-0.40	0.38	Ethyl esters 3½ to 5 c c	3	4th
	3	3		24-35	NR	0.31-0.44	0.38	Hydnocinol 4 c c Ethyl esters 2 to 3 c c	1	5th
	6	2	4	8-45	NR	0.11-0.13	0.12	Ethyl esters 3 to 5 c c	5	6th
					R ₁	0-39	0.39	Hydnocinol 3 c c	1	
	1	1		30	R	0.50	0.50	Ethyl esters 3½ c c	1	7th
	1		1	20	R	0.52	0.52	Ethyl esters 2 c c	1	8th
	1	1		19	NR	0.35	0.35	Ethyl esters 2 c c	1	9th
	1	1		47	R	0.39	0.39	Hydnocinol 2 c c	1	10th
	1		1	48	R	0.67	0.67	Ethyl esters ½ c c	1	13 days
	1	1		26	NR	0.72	0.72	Ethyl esters 2 c c	1	More than one month.

TABLE II (c)—*contd*

Types and sub types	Number of cases	SEX		Ages in years	Phase	Globulin range	Mean value	Preparation and doses	Number of cases	Days after injection
		Male	Female							
A ₂ (A ₂)	1		1	20	R	0.52	0.52	Ethyl esters 2 c c	1	8th
	1		1	35	NR	0.14	0.14	Hydnocreol 5 c c	1	13th
	1	1		25	NR	0.52	0.52	Ethyl esters 2 c c	1	More than one month.
C ₁ (B ₁ and B ₁ -A ₂)	6		6	13-16	NR	0.10-0.12	0.11	Ethyl esters 1 to 5 c c	6	4th
	5	4	1	25-50	NR ₄	0.08-0.17	0.13	Hydnocreol 6 to 10 c c	3	5th
					R ₁	0.81	0.81	Ethyl esters ½ to 3 c c	2	
	1	1		28	NR	0.11	0.11	Hydnocreol 3 c c	1	6th
	3		3	22-38	R	0.35-0.62	0.58	Ethyl esters ½ to 2 c c	2	7th
								Hydnocreol 5 c c	1	
	7	2	5	19-51	R ₃	0.65-0.80	0.73	Hydnocreol 1 to 7 c c	4	8th
					NR ₄	0.09-0.11	0.10	Ethyl esters 1 to 3 c c	3	
	2		2	29-40	NR	0.54-0.70	0.62	Hydnocreol 6 c c Ethyl esters 1½ c c	1 1	9th to 11th days
	6	6		23-52	NR	0.12-0.22	0.17	Hydnocreol 2 to 5 c c	All	12th
	9	2	7	24-48	NR ₄	0.09-0.11	0.10	Hydnocreol 1 to 4 c c	4	13th day to one month
					R ₅	0.52-0.95	0.74	Ethyl esters ½ to ½ c c	5	
	4		4	38-60	NR	0.11-0.58	0.35	Hydnocreol 2 to 10 c c	4	More than one month

TABLE II (c)—*contd*

Types and sub- types	Number of cases	SEX		Ages in years	Phase	Globulin range	Mean value	Preparation and doses	Number of cases	Days after injection
		Male	Female							
C ₂ (B ₃ and B ₋ A ₂)	4	1	3	8—20	NR	0.12—0.14	0.13	Hydnocreol 10 c c Ethyl esters 3½ to 4½ c c	1 } 3 }	4th
	2	2		25—67	NR	0.16—0.18	0.17	Hydnocreol 6 to 10 c c	2	5th
	6	6		14—50	NR	0.11—0.15	0.13	Hydnocreol 2 to 10 c c	6	6th
	11	8	3	20—70	NR ₈	0.19—0.31	0.25	Hydnocreol 5 to 10 c c	11	8th
					R ₁	0.58—0.73	0.66			
	3	1	2	39—50	NR ₂	0.12—0.15	0.14	Hydnocreol 3 to 6 c c	3	9th to 11th day
					R ₁	0.77	0.77			
	1		1	30	R	0.59	0.59	Hydnocreol 4 c c	1	7th
	2	2		34—52	NR	0.17—0.24	0.21	Hydnocreol 5 to 10 c c	2	12th day
	7	3	4	25—56	NR ₄	0.09—0.16	0.13	Hydnocreol 2 to 10 c c	7	13th day to 1 month
					R ₃	0.68—0.89	0.79		7	
	8	4	4	14—56	NR ₄	0.08—0.85	0.47	Hydnocreol 2 to 10 c c	7	More than one month
					R ₄	0.22—0.90	0.56	Ethyl esters 3 c c	1	

TABLE II (c)—concl'd

Types and sub types	Number of cases	SEX		Ages in years	Phase	Globulin range	Mean value	Preparation and doses	Number of cases	Days after injection
		Male	Female							
C ₃ (B ₃ and B ₃ -A ₁)	4	3	1	17-42	NR ₂	0.08-0.20	0.14	Ethyl esters 3 to 6 c c	2	5th day
					R ₂	0.18-0.26	0.22	Hydnocrocol 2 to 6 c c	2	
	2	2		28-34	NR	0.14-0.15	0.15	Hydnocrocol 3 to 10 c c	2	6th day.
	5	3	2	19-34	NR ₁	0.21-0.38	0.30	Hydnocrocol 5 to 10 c c	3	8th day
					R ₂	0.48-0.88	0.68	Ethyl esters $\frac{1}{2}$ c c	2	
	3	2	1	28-45	NR	0.15-0.19	0.17	Hydnocrocol 2 to 6 c c	3	9th day
	1	1		38	NR	0.23	0.23	Hydnocrocol 5 c c	1	12th day
	3	2	1	27-55	NR ₂	0.17-0.24	0.21	Hydnocrocol 3 to 10 c c	2	13 days to 1 month
					R ₁	0.68	0.68	Ethyl esters 3 c c	1	
	3	2	1	32-55	NR	0.24-0.51	0.38	Hydnocrocol 6 to 7 c c	3	More than one month

N B—Ethyl esters=Ethyl esters of *Hydnocarpus wightiana*, NR=Non reacting, R=Reacting

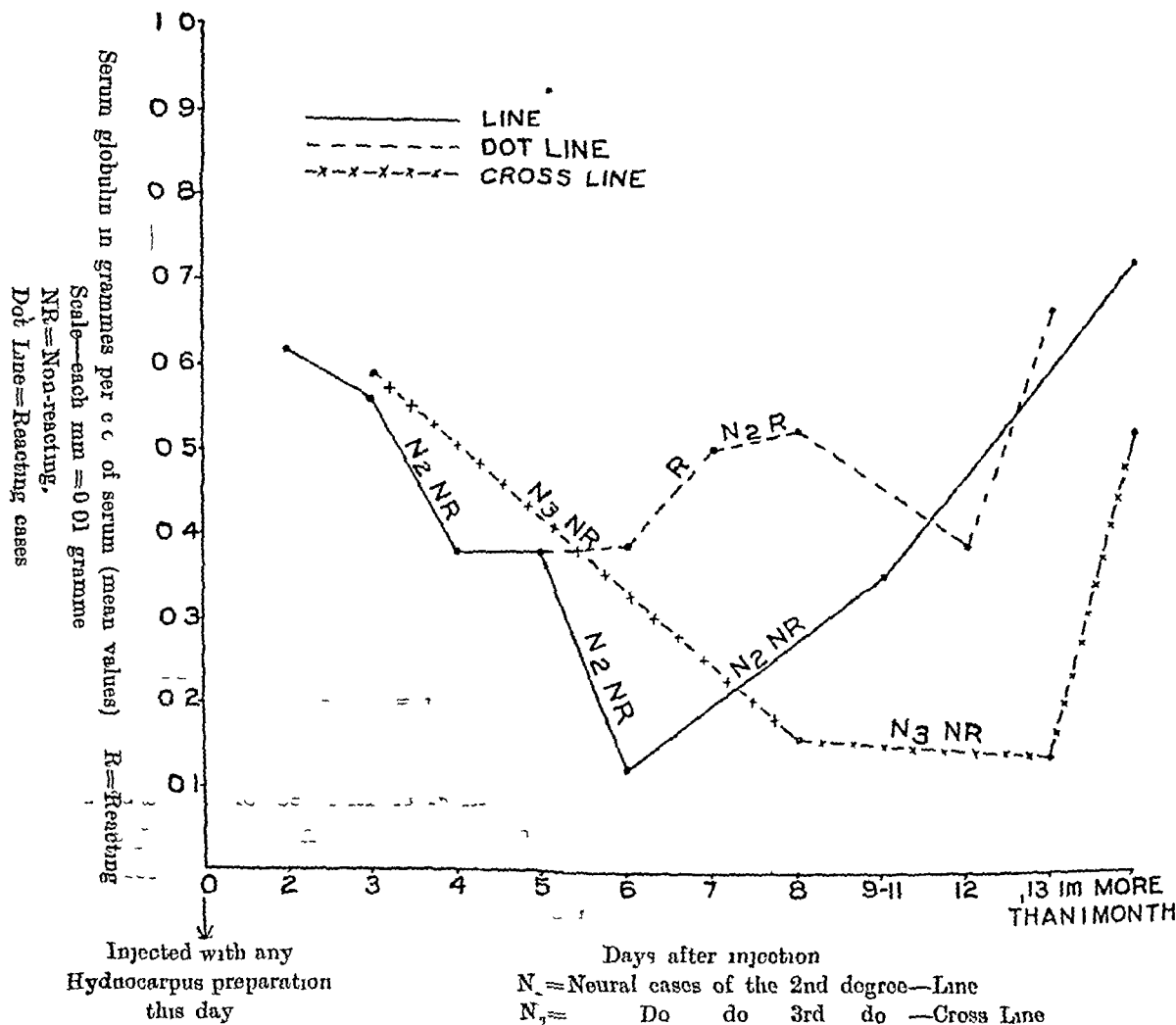
The quantitative changes in the serum globulin after an injection can also be represented graphically by a curve, and such curves for the different sub-types of cases are given in 2 graphs, one for the neural type of cases and the other for the cutaneous type of cases (see Graphs 1 and 2)

A reference to Graph 1 below will show that N₂ cases show a fairly high mean globulin value of 0.62 gramme on the 2nd day after an injection of any *Hydnocarpus* preparation and on the 3rd day after the injection, both N₂ and N₃ cases show practically the same high mean globulin values (0.57 and 0.59 g respectively). After the 3rd day, the fall in globulin value begins, and this fall is more gradual in N₃ cases. In N₂ cases on the other hand the mean globulin value seems to be stationary at a particular level from the 4th to the 5th day. Thereafter the further changes seem to depend upon whether the individual gets a lepra reaction or

not (a) If a reaction ensues, then the globulin value begins to increase, but this increase does not seem to reach the same height as reached on the 2nd day after the injection. This means probably that the reactions which set in late after an injection, i e, on the 5th day or thereafter, tend to be comparatively milder, with

GRAPH 1

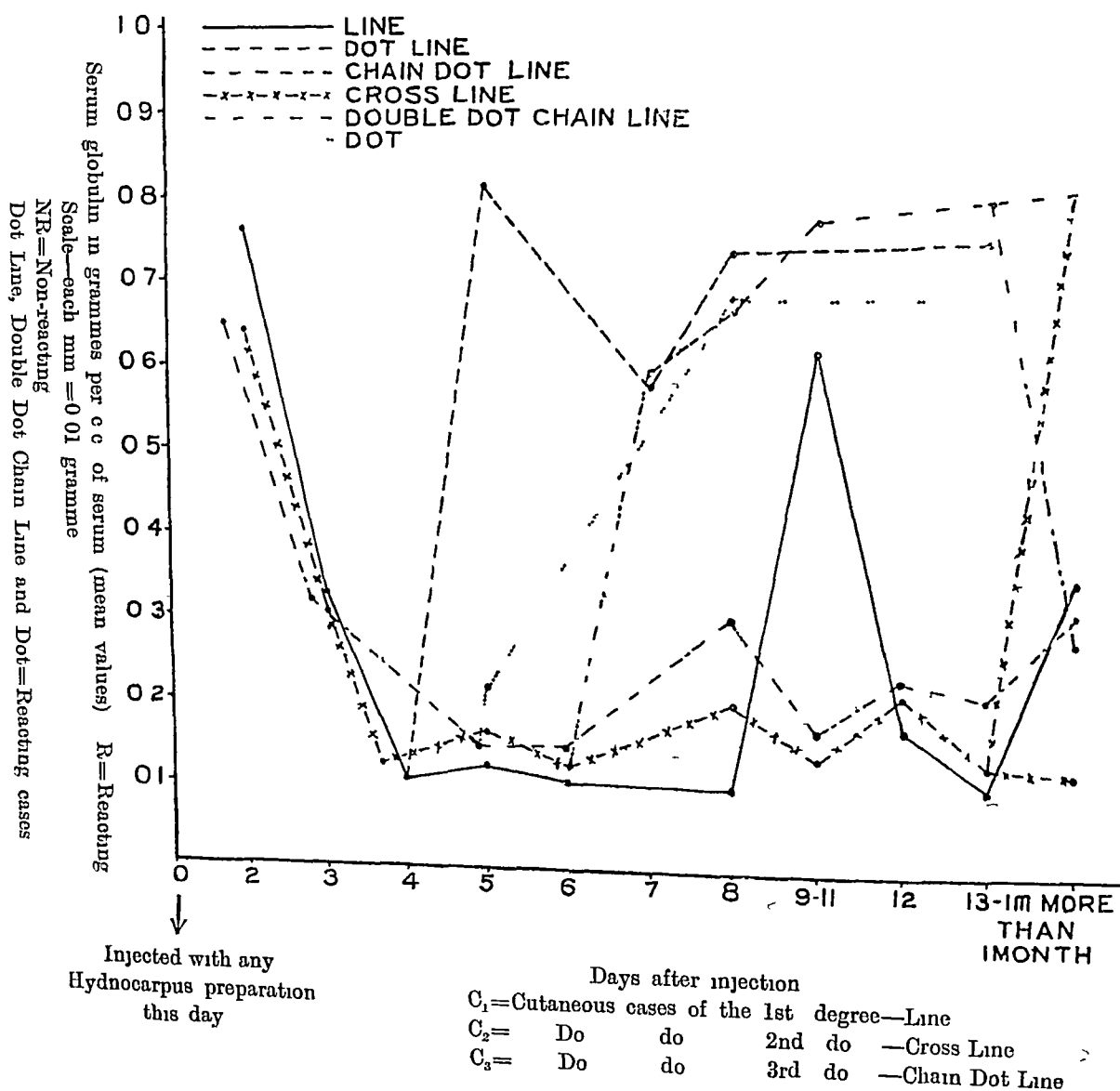
Neural cases



very few exceptions, than those that set in earlier, i e, on the 2nd or 3rd day. Clinical experience, of course, bears this out. (b) If no reaction occurs, then the globulin value begins progressively to decrease in both N₂ and N₃ cases, more rapidly in the former and fairly gradually in the latter. On the 6th day, N₂ cases

have reached the lowest value and thereafter a gradual rise in the globulin value is seen. But N_3 cases take longer to reach the lowest value, which they seem to do on the 8th day after the injection. The manner in which the globulin value rises again

GRAPH 2

Cutaneous cases

also seems to differ in the two degrees of neural cases. In N_2 cases, it is gradual and the tendency to rise is manifest even on the very next day after the lowest value is reached, whereas in N_3 cases it is rather abrupt and the rise begins only after the

low value has lasted for at least five days or so. This difference in the manner and time of rise in the globulin value after an injection in the two degrees of cases may be accounted for by the greater natural resistance of the N_2 cases, who do more exercise and lead more active lives than the deformed N_3 cases, whose level of natural resistance is considerably below par.

A reference to Graph 2 above will show that all the three degrees of cutaneous cases show a high mean globulin value (C_1 cases showing the highest value), on the 2nd day after an injection of any *Hydnocarpus* preparation. But, thereafter, all the three degrees of cutaneous cases show a fairly rapid and continuous fall in the globulin value up to the 4th day. After this, the further changes in the serum globulin value seem to depend upon whether a lepra reaction occurs or not. (a) If a lepra reaction sets in, the globulin value increases to a markedly high level, in fact it seems to be slightly more than what was reached on the 2nd day after the injection. This marked rise in the globulin value in reacting cutaneous cases seems to be maintained for a certain period which varies from three to six days, with an occasional slight reduction. Such comparatively long sustained high globulin values are not found in reacting neural cases, unless they have very severe and prolonged reactions. This means that, comparatively speaking, cutaneous types of cases tend to show more severe and prolonged reactions than neural cases. A further study of the three curves shows that the earlier the reaction sets in after an injection the greater is the globulin value and the longer this high globulin value is maintained. For example, C_1 cases, in whom a reaction has set in, on the 4th day, after the injection, show the highest globulin value on the very next day after the reaction has set in, and though this value falls slightly on the 7th day, still, it soon rises again within the course of a day to a slightly lesser height than before, and thereafter the high value is maintained practically up to the 13th day or so. Reactions setting in, on the 5th and 6th days after the injection in C_3 and C_2 cases respectively seem to run a parallel course. In fact, it is noteworthy that all the three degrees of reacting cutaneous cases run a more or less parallel course. Also, the late reactions that have set in, in C_2 and C_3 cases, have reduced the period during which a high globulin value is maintained. This means that such later reactions are comparatively milder and of a shorter duration. Clinical experience, with few exceptions, corroborates this. (b) If no reaction occurs then all the 3 sub-types of cases run a more or less identical course up to the 6th day, with a very slight increase in the globulin value on the 5th day in C_1 and C_2 cases only. From the 6th to the 8th day C_2 and C_3 cases show a tendency to rise, and the rise is complete on the 8th day. But C_1 cases on the other hand show a tendency to very slight reduction up to the 8th day, followed by a very abrupt rise. This abrupt rise is accompanied by an equally abrupt fall in the globulin value from the 11th to the 12th day. The significance of this sudden rise in the globulin value, in some cases at least of the C_1 type, on the 8th day after the injection is not sufficiently clear. Possibly such

cases might have been passing through a very mild and clinically unrecognizable reaction. There is no reason to suppose that this abrupt rise is a natural attempt to reach the normal level after the fall. For, if such had been the case, then there would not have been the quickly succeeding fall. In these cases, if another injection is given on the 8th day, possibly very severe and prolonged reactions may result. Clinical experience shows that some C_1 cases treated by weekly injections of Hydnocreol, do not manifest any pronounced clinical signs or reaction for 3 or 4 weeks, or even longer, but, thereafter, the very next injection produces a pretty severe and prolonged reaction, which sets in on the same day the injection is given. In these cases, a study of the globulin value curve week by week may show that very probably a succession of clinically unrecognizable reactions, in the form of a moderate increase in the globulin value on the 8th day after each injection, occurs. Finally when the last injection causes a still further increase in the globulin value, the proverbial 'last straw' seems to come into play and an explosive type of reaction results within 24 hours of the injection.

From the 12th day onwards after an injection there is a slight fall in the globulin value in all the three degrees of cutaneous cases, which is followed, after a variable period, by a tendency to rise.

Summary and conclusions

It is evident from a close study of both the graphs that the globulin value in lepers is not stationary. The effect of treatment with Hydnocarpus preparations seems to be to cause an immediate and very marked increase in the globulin value in both the cutaneous as well as the neural types of cases. This increase in the globulin value is followed by a fairly rapid fall in cutaneous cases and by a more gradual fall in neural cases. This fall continues up to a certain period which differs in the two main types of cases, viz., cutaneous and neural. After this period the further changes in the globulin value seem to depend upon whether a lepra reaction sets in or not. If a reaction occurs, then the globulin value rapidly mounts up in cutaneous cases, and more slowly and to a lesser extent in neural cases. This high rise in the globulin value is more or less maintained for a variable period, from 3 days to a week or more in cutaneous cases, but not so long maintained in neural cases, with very few exceptions.

These findings interpreted clinically mean —

(i) that, other conditions of treatment being identical, prolonged and severe lepra reactions are comparatively more frequent in cutaneous cases than in neural cases, (ii) that, with very few exceptions, the later the onset of the reaction after an injection with any Hydnocarpus preparation, the milder and shorter it tends to be, (iii) that clinically unrecognizable reactions, in the form of a moderate increase in the serum globulin, on the 8th day after an injection occur in some cutaneous

cases of the first degree, and possibly, to some extent, in other degrees of cutaneous cases also, and (*iv*) that a succession of such clinically unrecognizable reactions on the 8th day after each injection may finally tend to develop suddenly into an explosive and prolonged reaction, which sets in within a few hours after the receipt of a further weekly injection. The preceding injections of *Hydnocarpus* may, in this respect, be compared to the sensitizing doses of a foreign protein, and the last injection that precipitated the reaction to the exciting dose.

The formaldehyde test in leprosy

This test was done in all the cases studied including the healthy controls on the same day as the albumin and globulin estimations were carried out, using the well-known technique of Napier. One further modification in reading the results was introduced to suit this present experiment, viz., the actual degrees of coagulation present, in relation to the time of observation, were estimated by certain rough standards chosen by the writer and strictly adhered to throughout the experiment. Similarly the degree of opacity present, in relation to the time of observation, was estimated by attempting to read printed matter through the tube, both in direct as well as in indirect light, the printed matter used being the same throughout the experiment, and being held at the same position and distance from the tubes.

An analysis of the results shows at the outset that, except in very rare cases which will be discussed later, the aldehyde reaction in uncomplicated cases of leprosy of all types and stages is never positive, if the criteria of positivity strongly insisted upon by Napier (1927), in reading the results, are strictly followed. An analysis of the results of the formaldehyde test in some cases of leprosy is given below in Table III.

A study of the figures in this table shows that —

(i) The formalin coagulation reaction seems to bear no definite relationship with the globulin value, as both cases with high values as well as those with low values show the same degree of coagulation within the same time, (ii) there seems to be no definite connection between coagulation and opacity, thus cases showing the same degree of coagulation within the same time show different degrees of opacity, and (iii) the formalin coagulation reaction does not seem to bear any definite relationship with lepra reactions, as most of these cases were non-reacting when examined and as the test was completely negative in numerous truly reacting cases, who showed very much delayed coagulation and therefore are not included in Table III below.

Very hopeful opinions concerning the value of this test in leprosy, expressed by Sir Leonard Rogers, are unfortunately not substantiated by the results of this study.

TABLE III

Types and sub types of cases	Mean globulin value in g per c c	Phase	Degree of coagulation in relation to time	Degree of opacity in relation to time
C ₃ (B ₃ -A ₂)	0 10	NR	Full in 5 min	One plus in 5 min
C ₂ (B ₂ -A ₂)	0 13	NR	Full in 5 min	One plus in 5 min
C ₃ (B ₃ -A ₂)	0 14	NR	Full in 5 min	± in 5 min
C ₁ (B ₁ -A ₂)	0 95	NR	Full in 5 min	One plus in 5 min
C ₂ (B ₂ -A ₂)	0 11	NR	Full in 10 min	One plus in 10 min
C ₂ (B ₂ -A ₂)	0 18	NR	Full in 10 min	One plus in 10 min
C ₁ (B ₁ -A ₂)	0 80	NR	Full in 10 min	± in 10 min
C ₃ (B ₃ -A ₂)	0 77	NR	Full in 10 min	± in 10 min
C ₂ (B ₂ -A ₂)	0 85	NR	Full in 10 min	Nil in 10 min
C ₁ (B ₁ -A ₂)	0 50	NR	Full in 10 min	Nil in 10 min
C ₁ (B ₁ -A ₂)	0 16	NR	Full in 10 min	One plus in 10 min
C ₂ (B ₂ -A ₂)	0 31	NR	Full in 30 min	One plus in 30 min
C ₃ (B ₃ -A ₂)	0 22	NR	Full in 30 min	Nil in 30 min
C ₁ (B ₁ -A ₂)	0 13	NR	Full in 30 min	One plus in 30 min
C ₂ (B ₂ -A ₂)	0 76	NR	Full in 30 min	Nil in 30 min
C ₃ (B ₃ -A ₂)	0 61	NR	Full in 30 min	Nil in 30 min
C ₂ (B ₂ -A ₂)	0 40	NR	Full in 30 min	Nil in 30 min
C ₃ (B ₃ -A ₂)	0 88	NR	Full in 30 min	Nil in 30 min
C ₃ (B ₃ -A ₂)	0 35	NR	Full in 30 min	One plus in 30 min
C ₂ (B ₂ -A ₂)	0 14	NR	Full in 1 hr	One plus in 1 hr
C ₂ (B ₂ -A ₂)	0 15	NR	Full in 1 hr	One plus in 1 hr
C ₂ (B ₂ -A ₂)	0 15	NR	Full in 1 hr	Nil in 1 hr
C ₂ (B ₂ -A ₂)	0 16	NR	Full in 1 hr	± in 1 hr
C ₂ (B ₂ -A ₂)	0 12	NR	Full in 1 hr	± in 1 hr
C ₁ (B ₁ -A ₂)	0 09	NR	Full in 1 hr	One plus in 1 hr
C ₃ (B ₃)	0 12	NR	Full in 1 hr	One plus in 1 hr

± = Doubtful

N B—As the rest of the cases showed still further delayed coagulation, they are not reported here in the interests of space Min = Minutes Hr = Hour

A few cases show weak but definitely positive reactions (reported in the table above) and these were referred to Dr Napier for opinion. In a private communication through his assistant Dr C R Das Gupta, he expressed the opinion that such cases are the exception rather than the rule, and they are worth watching for signs of transient *Leishmania* infections. With this opinion, the writer is inclined to agree seeing that out of about 252 cases studied, only 7 or 8 of such cases have occurred, and they have shown no definite relationship with either the globulin value or the lepra reactions.

SUMMARY

(1) The results of a study into the serum proteins in 252 cases of leprosy, with special reference to Hydnocarpus treatment and in 56 healthy controls, are recorded in this paper.

(2) Serum albumin shows a significant reduction in lepers as compared with healthy controls and this reduction is probably caused by leprosy *per se*.

(3) Lepers of all types, except at certain phases, have a considerably higher globulin value than the healthy controls and the untreated leper controls.

(4) The effect of an injection of any Hydnocarpus preparation seems to be a marked increase in the globulin value in all types of cases detectable on the 2nd day after the injection.

(5) The further changes in the serum globulin on other days after the injection are described, and a certain difference in the behaviour of the globulin value in the two main types of lepers, viz, cutaneous, and neural, has been noted.

(6) The formaldehyde test in uncomplicated cases of leprosy is never positive, and there seems to be no definite relationship between formalin coagulation and serum globulin in lepers or lepra reactions.

ACKNOWLEDGMENTS

My grateful thanks are due to —

1 Dr M Wardman, M B, Ch B, Chief Physician, Purulia Leper Colony, for the facilities provided.

2 Drs E Muir and H W Wade, for their valuable criticisms and helpful suggestions.

3 Dr J M Henderson, for the sedimentation test figures in healthy controls supplied to me for comparative purposes.

4 Messrs Gopala Rao and Andrew, my laboratory assistants, for their very valuable help.

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THE EARLY STAGES OF SOME INDIAN MOSQUITOES *ORTHOPODOMYIA*

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[Received for publication, October 27, 1931]

IN the following descriptions of the larvae of the Indian species of *Orthopodomyia* the nomenclature adopted by Edwards and Given (1928) for the five important pairs of hairs on the clypeus has been followed. The letters *A*, *B*, *C*, *d*, and *e*, are used for these hairs and their position will be understood by referring to Fig 4 on Plate LIV. The hairs *A*, *B*, and *C* appear to be homologous with the frontal hairs of *Anopheles* larvae, *d* with the posterior clypeal hair, and *e* with the inner sutural hair. The inner and outer anterior clypeal hairs of *Anopheles* larvae appear to be represented in *Culicine* larvae by one pair of minute hairs situated externally and slightly dorsally to the preclypeal spines. In some *Culicine* larvae hairs *B* and *C* stand directly one in front of the other, in such cases the anterior hair is called *B* and the posterior *C*. In the larvae of several genera hair *d* is very small and difficult to see, but in the larvae here described it is well developed.

The fourth stage larvae of the Indian species of *Orthopodomyia* may be fairly easily distinguished from those of other genera by the following characters: siphon without pecten, a single pair of hair tufts situated at about the middle of the tube, comb with a row of large teeth and with a second row of smaller fanged teeth, some long single hairs on the abdomen, antennal shaft smooth, tuft at one-quarter or one-third from the base. In *O. anopheloides* and *O. flavicosta* there is a chitinized saddle on the 8th abdominal segment and in the former there is a well-developed saddle on the 7th segment also, but in *O. flavithorax* there is no very definite saddle on either segment, only some indefinite chitinization on the 8th, as indicated in Fig 8.

The pupae do not show any marked modifications, respiratory horn of moderate length, wide at the apex in side view, dendritic tuft on abdominal segment 1 well developed, one of the five submedian and sublateral hairs on tergites 4 to 6

fairly long or very long, tufted lateral hairs on segments 7 and 8, paddles nearly twice as long as broad, rather flattened on the outer side towards apex, apical half very transparent, a short terminal hair, moderately developed midrib, an irregular dark marking across the base

Key to the 4th stage larvae of the Indian species of *Orthopodomyia*
(See Plate LIV)

- | | | |
|---|---|------------------------|
| 1 | 7th abdominal segment with a large chitinized saddle (Fig 2), one branch of inner subdorsal hairs of anal segment much longer than the others (Fig 2), a separate lateral chitinized strip at base of anal segment on each side (Fig 2) | <i>O. anopheloides</i> |
| | 7th abdominal segment without a chitinized saddle, all branches of inner subdorsal hairs of anal segment about the same length (Fig 8), no separate chitinized strip at base of anal segment | 2 |
| 2 | Larger comb teeth ending in a single long point (Fig 7), five pairs of clypeal hairs equally developed and with long plumose branches (Fig 14), hairs on sides and dorsum of thorax remarkably developed and with long plumose branches | <i>O. flavithorax</i> |
| | Larger comb teeth ending in several sharp points (Fig 6), five pairs of clypeal hairs not all equally developed, <i>d</i> being comparatively small with only 3 or 4 fine branches, <i>e</i> fine and single or split (Fig 10), hairs on dorsum of thorax fine and inconspicuous, lateral hairs only being well developed | <i>O. flavicosta</i> |

Key to pupae (See Plate LIV)

- | | | |
|---|--|------------------------|
| 1 | Longest hair on tergite 6 very long, reaching beyond the posterior margin of tergite 8, and either single or two branched (Figs 11 and 12) | 2 |
| | Longest hair on tergite 6 comparatively short, not reaching beyond posterior margin of tergite 7, and usually of four branches (Fig 1) | <i>O. anopheloides</i> |
| 2 | Longest hair on tergites 4 to 6 single (Fig 12) | <i>O. flavicosta</i> |
| | Longest hair on tergites 4 and 5 three branched that on tergite 6 two-branched (Fig 11) | <i>O. flavithorax</i> |

O. anopheloides Giles (*Mansonia*) Larva, 4th stage (Plate LIV, figs 2, 4, and 5) Head and antenna deep brown or black, clypeal hair *A* with 9 to 11 branches, more usually 10, *B* and *d* with 6 to 8 branches, the latter well developed, *C* with 4 to 6 branches, *e* fine and long, often split into two towards the tip, preclypeal spines fairly long, slender and tapering, antennal shaft about 0.41 mm long, stout on rather more than the basal half, tapering to the tip, tuft of 6 to 8 branches at one-third from the base, apical spines all at tip of shaft Thorax with lateral tufted hairs well developed and some long single hairs Abdomen usually reddish in colour, the first two segments with lateral tufted hairs, segments 3 to 8 with fine hairs some of which are single and long, segment 7 with a chitinized saddle covering rather more than the dorsal half in side view, a saddle on segment 8 nearly enclosing the segment, comb with 7 to 10 larger teeth and 25 to 30 smaller fringed teeth.

siphon dark brown or black, lighter at apex, 1 to 1.1 mm long and about 5 times as long as the diameter at base, tuft of 8 to 14 strong subplumose branches arising at about the middle of the tube but very slightly nearer the base than the apex, anal segment enclosed in a chitinized ring, a separate chitinized strip on each side at the base, outer subdorsal hairs single and long, inner split into a number of branches one of which is much longer than the others, lateral hair with two or three fine branches, ventral pair of anal papillae about half the length of the dorsal, the latter rather more than half the length of the anal segment, anal fan well developed, the hairs arising from a fan plate and each splitting into a number of branches some distance from the base

Pupa (Plate LIV, figs 1, 3 and 9) this may be distinguished by the characters given in the key, the innermost submedian hair on tergites 3 to 7 is very small, whereas in the other two species it is larger and spine-like

Habitat tree-holes during the monsoon, found in the Western and Eastern Himalayas up to 8,000 feet, along the foothills to Bengal and Assam, in the Western Ghats from North Kanara to Malabar and in Ceylon

O. flavithorax Barraud *Larva*, 4th stage (Plate LIV, figs 7, 8, 13 and 14) this has a very characteristic appearance even to the naked eye, owing to the remarkable development of the hairs on the head and thorax. Hairs *A*, *B*, *C*, *d*, and *e*, on the clypeus are all about equally developed and each has a number of subplumose branches which are as long, or longer, than the clypeus, antennal shaft about 0.58 mm long, stout on the basal one-third, more slender apically, brown for the most part, paler at tip, tuft of about 5 subplumose branches, arising at about one-quarter from the base, apical bristles all very near tip of shaft, preclypeal spines long, slender, tapering. Thorax densely clothed dorsally and laterally with long tufted hairs with subplumose branches. Abdominal segments with some very long single or two-branched hairs, as well as shorter tufted hairs with subplumose branches, segment 7 without a chitinized saddle, segment 8 with indefinite chitinization as indicated in Fig 8, not forming a definite saddle, comb with 10 to 12 larger teeth and 18 to 20 smaller delicately fringed teeth, siphon from 1.4 to 1.5 mm long and 5 or 6 times the length of diameter at base, dark brown, a very narrow darker ring at base, paler at extreme tip, hair tuft of about 8 finely frayed branches, arising at about two-fifths of the length from the base, anal segment enclosed in a chitinous ring, no separate chitinization at base, outer subdorsal hairs single and long, 6 or 7 times the length of the chitinized part of the segment, inner pair split into a number of branches all about the same length and about two and a half times the length of the segment, ventral anal papillae about half the length of the dorsal pair, the latter only about half the length of the anal segment, lateral hair of 2 or 3 fine branches, anal fan well developed, about 14 hairs arising from fan plate, each hair split some distance from the base into a number of fine branches

Pupa (Plate LIV, fig 11) distinguished by the presence of long three-branched hairs on tergites 4 and 5, and a very long two-branched hair on tergite 6

Habitat tree-holes during the monsoon, North Kanara and Malabar, near sea-coast

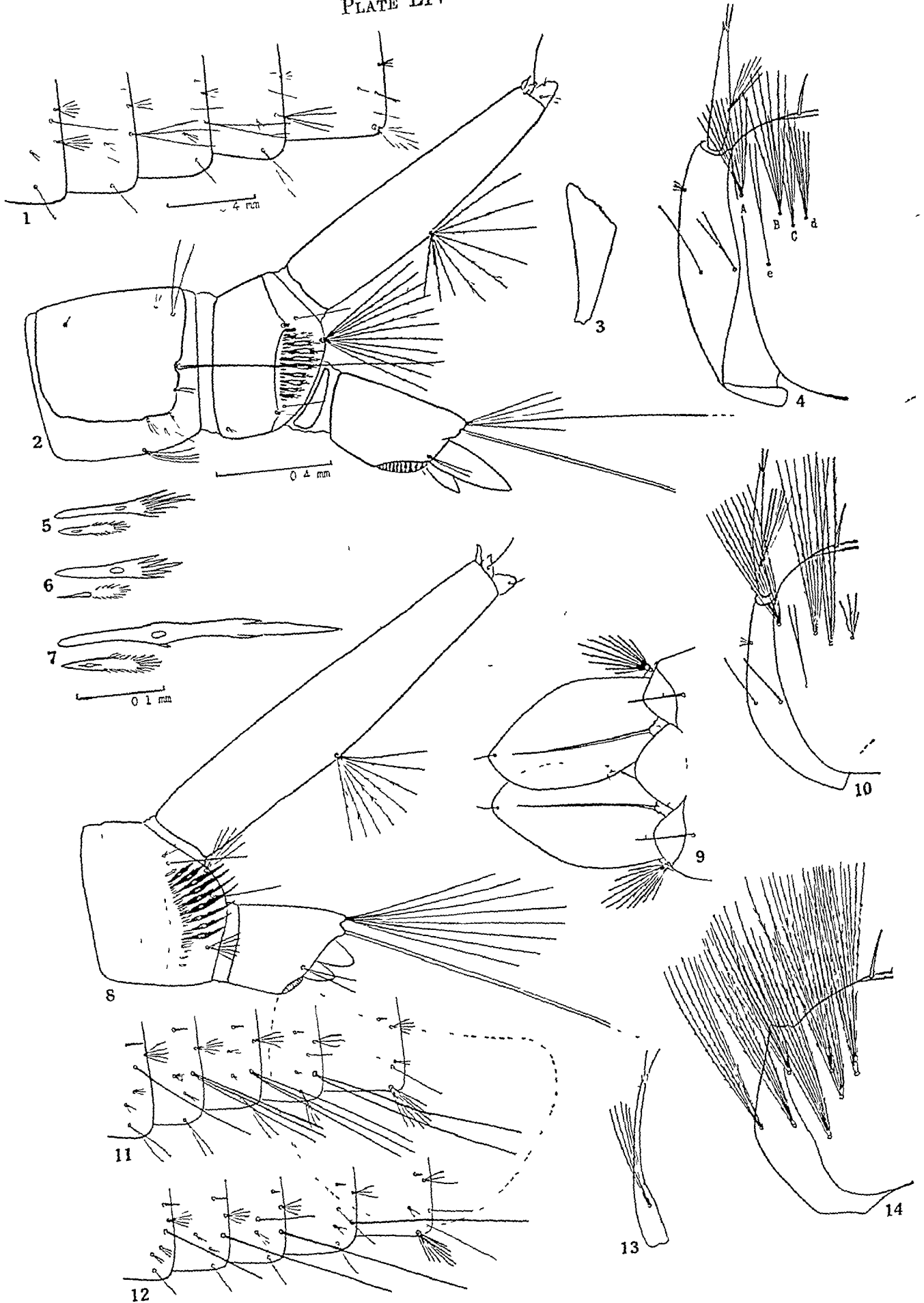
O. flavicosta Barraud *Larva*, 4th stage (Plate LIV, figs 6 and 10) this larva differs in several respects from that of *O. flavithorax*, with which it is found breeding in association, the hairs on the head and thorax being much less developed, and the larger comb teeth being of different form. Head yellowish, slightly darker posteriorly, antennal shaft about 0.5 mm long tapering from base to apex, paler on apical part than towards base, tuft of about 6 branches, arising at a point between one-quarter and one-third of length of shaft from base, clypeal hair *A* usually of 9 branches, variation 9 to 12, *B* of 6 or 7 branches, *C* of 4 or 5, *d* of 3 or 4 fine branches much shorter than *B* or *C*, *e* fine and usually single but may be split into two towards the tip, preclypeal spines fairly long and tapering, pale in colour. Thorax with lateral tufted hairs well developed but hairs on dorsum fine and inconspicuous. The first two abdominal segments with lateral tufted hairs, the following segments with fine hairs, some of which are long and single as usual, 7th segment without a chitinized saddle, 8th segment with a definite saddle nearly enclosing the segment, comb with 7 to 10 larger teeth and about 20 smaller fringed teeth, the larger teeth end in a number of sharp points and resemble a hand, there being usually 5 flattened leaf-like points, siphon 1.2 mm long, 5 or 6 times the length of the diameter at the base, hair tuft of about 6 branches, finer than in the other two species here described, arising very slightly nearer the base than the apex of the tube; anal segment very similar to that of *O. flavithorax*.

Pupa (Plate LIV, fig 12) distinguished by the characters given in the key, except for these it is very similar to that of *O. flavithorax*.

Habitat tree-holes during the monsoon, North Kanara, near sea-coast

REFERENCE

EDWARDS F W, and GIVEN, D H C *Bull Ent Res*, **18**, p 338
(1928)



EXPLANATION OF PLATE LJV

Drawings illustrating points of structure of diagnostic importance of the 4th stage larvae, and pupae, of Indian species of *Orthopodomyia*

Figs 1, 9, 11 and 12 drawn to the scale shown under Fig 1

Figs 2, 3, 4, 8, 10, 13 and 14 drawn to the scale shown under Fig 2

Figs 5, 6 and 7 drawn to the scale shown under Fig 7

Fig 1	<i>O anopheloides</i>	Pupa showing hairs on dorsum of one side on abdominal segments 3 to 7 (segt 7 on right)
„ 2	<i>O anopheloides</i>	Larva terminal segments showing chitinized plates on segments 7 and 8, comb, siphon, and anal segment
„ 3	<i>O anopheloides</i>	Pupa respiratory horn, side view, from flattened preparation
„ 4	<i>O anopheloides</i>	Larva one side of head showing hairs on dorsum, drawn from cast skin, mouth brush omitted
„ 5	<i>O anopheloides</i>	Larva two comb teeth enlarged
„ 6	<i>O flavicosta</i>	Larva two comb teeth enlarged
„ 7	<i>O flavithorax</i>	Larva two comb teeth enlarged
„ 8	<i>O flavithorax</i>	Larva terminal segments showing indefinite chitinization on segment 8, comb, siphon, anal segment
„ 9	<i>O anopheloides</i>	Pupa extremity of abdomen
„ 10	<i>O flavicosta</i>	Larva one side of head showing hairs on dorsum, mouth brush omitted
„ 11	<i>O flavithorax</i>	Pupa showing hairs on the dorsum of one side on abdominal segments 3 to 7 (segt 7 on right)
„ 12	<i>O flavicosta</i>	Pupa showing hairs on the dorsum of one side on abdominal segments 3 to 7 (segt 7 on right)
„ 13	<i>O flavithorax</i>	Larva antenna
„ 14	<i>O flavithorax</i>	Larva one side of head showing hairs on dorsum, mouth brush omitted

A NOTE ON THE TYPE OF CHOLERA BACTERIOPHAGE ISOLATED FROM CASES DURING A SMALL EPIDEMIC OF CHOLERA IN MADRAS

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THE existence of different types in cholera bacteriophages, e g, types A, B and C, was first demonstrated by Asheshov and his collaborators (1930). Of these, the A type of 'phage is the quick acting type acting on smooth cultures alone, while B and C types are slow acting 'phages which act on rough cultures as well. It was also noted by them that on the basis of the lytic action demonstrated by an 'A' type of bacteriophage the various cholera vibrios so far obtained from different parts of India fell into 4 groups, and that, while no bacteriophage was isolated that was able to lyse spontaneously all the four groups of vibrios, any one type could be adapted to lyse, after suitable laboratory cultivation, all the remaining groups of vibrios. They noted, 'It was found that type A cholera-bacteriophage isolated at Patna acted only on Groups I and II some of them acting only in Group I. At Puri, a bacteriophage was found acting on Groups I, II and IV of vibrios but of a feeble virulence. As no stools were examined in Madras no bacteriophage spontaneously acting on Group III was isolated.'

(Report to the Indian Research Fund Association, 1930)

We had an opportunity to study this question during a small epidemic of cholera in Madras city in April and May 1931, when a few cases were admitted to each of the two infectious diseases hospitals in the City. We examined the stools of 15 cases in all for the presence of cholera bacteriophage, and from 12 of them an 'A' type of cholera bacteriophage was isolated that showed lysis with a representative

strain of each of the four groups of vibrios noted above. This was therefore a polyvalent 'phage acting on all the types of vibrios known at present. This is the first instance on record, we think, when this type was found to be present in so many cases during an epidemic.

Asheshov's technique was followed in detail, which one of us had an occasion to study in his laboratory some time ago. About 2 c.c. of the liquid cholera stool were inoculated into 30 c.c. of Asheshov's medium in a flask (Papain broth pH 8.4). This was done in the hospital itself and the inoculated flasks were brought to the laboratory for further incubation. After 24 hours of incubation, the contents of the flasks were filtered through L3 candles, and the filtrate immediately tubed in ampoules. One ampoule was taken for determining the presence and the type of 'phage.

A separate peptone water culture was also made from the stool to isolate a strain of cholera vibrios to study the group to which it belonged.

The details of our findings are summarized in the following table. The 'phages exhibited a very low grade of virulence —

TABLE

Showing the details of vibrios and bacteriophages isolated

Number of culture	Group and character of vibrios	Type of 'phage	Date of isolation	Hospital
36	II	Polyvalent A	25-4-31	Krishnampet Isolation Hospital
38	I	Nil	26-4-31	Do
39	Rough	Polyvalent A	26-4-31	Do
40	Rough	Nil	26-4-31	Do
41	Rough	Polyvalent A	7-5-31	Infectious Diseases Hospital, Tondiarpet
42*	I	Do	7-5-31	Do
43	Rough	Do	7-5-31	Do
44	Rough	Do	7-5-31	Do
45	I	Do	7-5-31	Do
46*	Rough	Do	7-5-31	Krishnampet Isolation Hospital
47*	Non agglutinating	Do	7-5-31	Do
48	Non agglutinating	Do	8-5-31	Infectious Diseases Hospital, Tondiarpet
49	Non agglutinating	Do	8-5-31	Do
50	Rough	Do	8-5-31	Do

* Denotes fatal cases

Comments

It must be noted here that in the Infectious Diseases Hospital, Tondiarpet, a few cases of cholera had been treated by us in November and December 1930, the 'phage used being that supplied by Asheshov—a mixture containing polyvalent 'A' type and B and C types. Asheshov has shown that cholera 'phage, once administered to a case in the hospital, afterwards propagates itself 'naturally' in hospital cases, so that the same polyvalent 'phage can be recovered from a case admitted there even a month after the administration of 'phage ceased (*Personal communication*). It could therefore be contended that the finding of a polyvalent 'phage in the cases admitted to this hospital in the present series is merely due to such 'natural cultivation'. We doubt, however, if our present findings are to be accounted for in this way. The 'phage was used for the last time in treatment on 7-1-31. It is true that a case or two of cholera were admitted to the hospital daily since then. But in the present series, the polyvalent 'phage was isolated in that hospital on 7-5-31, i.e., after four months' interval. Again while the 'phage used for treatment contained all the three types, i.e., A, B and C, only A type of 'phage was isolated in the present series. Though types B and C are slow acting 'phages there was every chance of their being propagated in some of the patients at least.

A fairly conclusive evidence, however, is that a polyvalent phage was isolated from a few cases admitted to the Krishnampet Isolation Hospital which is situated in the southern part of the city at a distance of over seven miles from the former and where 'phage treatment had not been undertaken at all. There was no interchange of staff between the two hospitals.

REFERENCE

ASHESHOV, N *et al* (1930)*Ind Jour Med Res*, **17**, pp 971-984

A NOTE ON THE ANTIGENIC STRUCTURE OF SECONDARY CULTURES OBTAINED WITH THE THREE TYPES OF CHOLERA 'PHAGES AND A STRAIN OF CHOLERA VIBRIO.

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WHILE some information is now available regarding the changes which bacterial cultures undergo after homologous 'phage action, particularly with regard to changes brought about in their morphological and biochemical characters, the antigenic characters of such secondary cultures have not yet been sufficiently studied. Burnet (1930) summarizes these changes as follows: 1. Development of roughness. 2. Loss of heat-labile antigens. 3. Development of magglutinability, with power to produce normal agglutinins.

In connexion with the development of roughness Hadley (1927-28) has put forward the hypothesis that bacteriophagy is essentially a phenomenon of microbial dissociation and that 'phage resistant or secondary cultures are really the manifestations of a rough 'phage of the normal smooth culture and are almost identical with the rough variants which are obtainable in the entire absence of any bacteriophage action. A logical corollary to this hypothesis would be that the two types of rough strains so obtained would show some resemblance in their antigenic structure.

In this note some observations bearing on these points are recorded. The observations were made with one strain of cholera vibrio and the three types of cholera 'phages isolated by Dr. Asheshov.

Material and methods employed

1 *Types of cholera bacteriophage*—These were the three types described by Dr Asheshov (1930) and obtained from him, viz, 64 A, 146 B, and 13 C. They were cultivated and maintained in Asheshov's medium (papaine broth pH 8.4). Of these '64 A' type of phage was the quick acting type of phage but acting only on the smooth strains of vibrios and phages B and C were slow acting types acting on rough strains as well.

2 *Cholera vibrio J*—This was a smooth strain isolated in Patna by Dr Asheshov. As recommended by him its smooth characters were maintained by sub-culturing it whenever necessary, on neutral biomo-thymol blue papain agar. This strain was chosen because it was in constant use in the laboratory and had given consistent results. It was tested and found to be 'ultra pure', i.e., not contaminated with phage.

3 *Rough variant J*—This was obtained in the first instance by plating a three weeks old broth culture. A rough looking colony was picked out and in order to enhance its roughness if possible, it was grown again in alkaline broth to which was added its homologous antiserum in a dilution of 1 in 10. It was noted, however, that the rough character was not permanent and the culture had a tendency to revert to its smooth type, particularly on keeping. In all the experiments therefore fresh sub-cultures from rough colonies were used in order to eliminate the smooth elements as far as possible.

4 *Secondary cultures*—These were of three types corresponding to the three types of phages noted above. They were obtained by inoculating 10 c.c. of papaine broth with a suitable amount of five hours old smooth culture and one drop of the appropriate phage and incubating overnight. The secondary growths obtained were maintained on agar slopes previously smeared over with the corresponding phage.

5 *Agglutinating sera*—Four types of agglutinating sera were employed.

(a) Serum prepared by immunizing a rabbit against a suspension of smooth 'J' culture killed at 58° C. This would contain both the H and O agglutinins.

(b) Serum prepared against the same strain but with the culture heated, to 100° C for 2 hours. This would therefore contain only the O type of agglutinins.

(c) Three sera prepared with the three phage-resistant variants, i.e., secondary cultures of J killed at 58° C.

(d) Serum against the rough variant of J obtained as noted before and killed at 58° C.

All the rabbits were immunized by five intravenous injections with 1 c.c. stock suspensions at intervals of five days and the animals were bled nine days after the last injection.

Technique

The technique employed was more or less the same, as described by Balteanu in his work on the antigenic structure of the cholera vibrio. For the tests the

suspensions were made from cultures 24 hours old in 0.85 per cent saline (distilled water was used for the rough variant)

The strength of all the suspensions was brought to 1,000 millions per c c. One half of each was heated to 100° C (for 2 hours to destroy the 'H' agglutinin)

The tests were carried out in Dreyer's tubes arranged in series. 0.25 c c of the suspension was first added, and then an equal volume of various dilutions of the sera in 0.85 per cent saline.

The tubes were incubated in a water bath at 50° C for 3 hours and then kept overnight in an incubator at 37° C. The readings were taken the next morning. The titre and the nature of the agglutination were noted.

The results obtained are given below. Table I summarizes the titres obtained against the homologous cultures, all the secondaries and the rough variant with the two antisera *vs* J, one containing both 'H' and 'O' agglutinins and the other containing only the 'O' agglutinin —

TABLE I

Agglutination of normal culture J and its variants

Culture	ANTISERUM J (H O)		ANTISERUM J O (O)	
	Flagellar	Somatic	Flagellar	Somatic
J { Killed at 58° C for ½ hr Heated to 100° C for 2 hrs	1,600	1,600 1,600		1,600 1,600
J S A { Killed at 58° C for ½ hr Heated to 100° C for 2 hrs	200	? 50 incomp 50 "		50 incomp 50 "
J S B { Killed at 58° C for ½ hr Heated to 100° C for 2 hrs	800	800 800		800 800
J S C { Killed at 58° C for ½ hr Heated to 100° C for 2 hrs	400	400 400		400 400
J R { Killed at 58° C for ½ hr Heated to 100° C for 2 hrs	800	50 50		50 50

J = Normal smooth culture J S A = Secondary of J after the action of ch ϕ 64 A
J S B = Secondary after the action ch ϕ 146 B J S C = Secondary after the action ch ϕ 13 C
J R = Rough variant of J

It is seen that the secondary culture obtained after the action of A type of 'phage differs considerably in its antigenic make up from the normal smooth type and to a certain extent from the secondaries obtained with the other two types of 'phages. Thus the somatic antigen is very considerably altered in the case of secondary A and to a less extent in the case of secondaries B and C. While the titre obtained with the flagellar antigen is reduced to some extent in the case of all secondaries, the change is not so marked as in the case of the somatic antigen.

The rough variant on the other hand shows little reduction in the titre for the flagellar antigen. The somatic antigen, however, is changed to the same extent as in the case of secondary A. The rough variant therefore more or less approximates to the secondary A as far as its somatic antigen is concerned. While these observations were confirmed in the majority of tests done, there were a few instances when a spontaneous agglutination was noted with all the cultures put up, in all dilutions both with the immune sera and the normal control serum. Such occasionally variable results have also been noted by other workers, e.g., Wolfe (1930) who worked with Flexner dysentery bacillus and its secondary cultures.

The changes observed in the antigenic structure between the three types of secondaries might be correlated with the nature and rapidity of action of their homologous 'phages, and explain to a certain extent the differences in the behaviour of the three types of phages. Asheshov has shown that a secondary culture obtained with any one of the above types of 'phages was still lysable by the other two types of 'phages. Since B and C 'phages act on the rough strains also, their action on the secondary A is easily understood. But the 'A' type of 'phage also acts on the secondaries of B and C and since 'A' 'phage does not act on rough strains at all the secondaries of B and C would have to be considered, not as truly rough, but analogous to some extent in their somatic antigen with the normal smooth culture. That it is so, is borne out by the results obtained above. B and C secondaries retain their original somatic antigen to a considerable degree.

Further corroborative evidence is given by the absorption experiments described below and by the varying power of the three secondary cultures to provoke normal agglutinins.

Absorption tests—The antiserum J was absorbed separately by each of the three types of the secondaries and the agglutination titres of the absorbed sera were tested against the homologous culture, i.e., J. The following results were obtained.

It is seen that secondaries B and C absorbed almost all the agglutinins for antiserum *vs* J, but with the serum absorbed with the secondary A, granular agglutination to a titre of 1 in 200 was obtained. This again confirms the nature

and extent of the antigenic change in the secondary A, as compared with changes in secondaries B and C

TABLE II
Absorption tests

	SÉRUM AFTER ABSORPTION WITH			
	J	Secondary A	Secondary B	Secondary C
Culture J		200 g	<50	<50

In view of this change in the somatic antigen it was decided to see to what extent the secondary cultures would provoke normal agglutinins. Suitable antisera against all the secondary types were prepared as noted before and cross-agglutination tests were put up both with the normal smooth cultures and the secondary culture. The results are summarized in Table III —

TABLE III
Cross-agglutinations

	ANTISÉRUM AGAINST							
	J		J S A		J S B		J S C	
	Flagellar	Somatic	Flagellar	Somatic	Flagellar	Somatic	Flagellar	Somatic
J	1,600	1,600	100 in comp	100 in comp	200	200	1,600	1,600
J S A	200	50	200	200	100	50	200	50
J S B	800	800	100	100	400	400	400	400
J S C	400	400	100	100	400	400	1,600	1,600

It is seen that the power to provoke normal agglutinins was diminished to a great extent in secondary 'A' and to a less extent in the case of secondary B, while it more or less approximated to the normal type with secondary C.

Further work in this connexion will be undertaken when we have succeeded in obtaining a fairly stable rough culture. Experiments in that direction are in progress.

A reference might be made here to the work of Burnet (1929) who obtained from a smooth non-motile strain of *B. sanguinarum* (*Salmonella gallinarum*) both S and R resistant forms by suitable 'phage action. He found that when no change of 'phage (S or R) occurred after 'phage action, the resistant variants were serologically identical with their parent strains and that no change in the heat stable agglutinin was noticed, while our results are more or less in agreement with the above in respect of secondaries B and C, they are not so in the case of secondary A. It is to be noted, however, that the variants obtained by Burnet had been purified from 'phage by repeated platings. In the present work, the secondary cultures were not so purified and as has already been noted they were always sub-cultured on agar after the medium was smeared over with the corresponding 'phage.

It was in these conditions that a change in the somatic antigen was noticeable in the secondary cultures.

SUMMARY

The antigenic characters of the three types of secondary cultures were studied. The main change was in respect of the presence of the somatic antigen. This antigen was considerably diminished in the case of secondary A but it persisted in varying amounts with secondaries B and C. The above gradation was noted in the case of flagellar antigen also. The power to provoke normal agglutinins was found to be proportional to the amount of somatic antigen present. The results are in accordance with the difference in behaviour noted with the three types of cholera 'phages. The rough variant, produced independently of any phage action, showed more or less the same antigenic structure as was noted with the 'A' type of secondary culture.

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SOME FACTS ABOUT THE INCIDENCE OF SPLENOMEGALY IN BENGAL

BY

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[Received for publication, November 17, 1931]

IN the Tropics, especially in a province like Bengal, where malaria and kala-azar are so rampant, any enlarged spleen, which is detected by the average practitioner during the examination of his cases, is considered to belong to one or the other of the two above-mentioned conditions. In this short paper, the writer has tried to survey the incidence of splenomegaly in various conditions as met with in a large series of unselected cases. The figures and facts have been collected from the necropsy findings in the post-mortem examination room of the Medical College Hospital as well as from the records of the Pathology Department of the same college. The writer had an unique opportunity of studying each one of these cases which were obtained during the period under review, having been intimately connected with both the college and the hospital for more than 12 years.

During the period of 10 years, i.e., from 1920 to 1929, both years inclusive, the total number of necropsy examinations, which were obtained from the medical college group of hospitals, was 977. This series consisted of as large a variety of cases as one could expect to get from a general hospital. Out of this number, 525 cases, i.e., about 54 per cent, were found to have enlarged spleens, the weights of which varied from 200 grammes to 1,500 grammes and above. I have taken 200 grammes as the minimum weight of an enlarged spleen because, by a comparative study, I find the average weight of the spleen in apparently normal individuals as met with in medico-legal post-mortem examinations, to vary from 100 to 150 grammes. I have classified the degrees of enlargement as in the following table —

Number of cases	Weight in grammes
356	200 to 500
118	500 to 1,000
39	1,000 to 1,500
12	above 1,500

Out of these 525 cases, accessory spleens were detected in 26, i.e., about 5 per cent of the total number of cases of splenomegaly. No special significance could be attached to this finding except in a few cases where the accessory nodule was enlarged enough to appear as a palpable mass in the abdomen giving rise to diagnostic speculations.

In 203 cases, i.e., 21 per cent of the total number, the organs were felt to be fairly firm and on section various degrees of toughness were noticed. In almost all of them, the trabeculae were numerous and very prominent, appearing to the naked eyes as little areas of scarring. The colour of the organ varied from a dull red to deep chocolate, according to the cause of death. A very remarkable feature of this group was the frequency of the capsular changes. In all of them there were various degrees of generalized thickening of the capsule and in 198 cases, i.e., about 97 per cent, there was definite evidence of perisplenitis with adhesions to the neighbouring viscera. Contrary to the usual and common belief, these capsular changes and the adhesions were found to be very infrequent in cases of true leishmania—positive cases of kala-azar. Another feature of these cases was that the above changes did not depend on the relative size of the spleen. Some of the enormously big organs showed only little or localized changes whereas an organ of 200 to 500 grammes showed the most intensive hyaloserositis on the capsule and inseparable adhesions with the diaphragm, stomach and large intestine.

The 525 cases are grouped as follows according to the various diseases which were diagnosed during life —

Malaria	31	Hodgkin	1
Kala-azar	67	Heart disease	38
Tuberculosis	88	Acute infection	104
Portal cirrhosis	19	Miscellaneous	148
Leukæmia	3	Unknown	26

The examination of the cases of malaria and kala-azar in detail reveals interesting feature. It shows how in a province like Bengal, the supposed endemic home of malaria and kala-azar, the greatest difficulty is experienced in the accuracy of diagnosis and determination of the ætiology of the splenomegalic condition in life. Out of the 98 cases (31 cases of clinically diagnosed malaria and 67 cases of kala-azar), in at least 24 cases, this was definitely noticed. From clinical signs and symptoms, they were diagnosed, either as malaria or kala-azar, but necropsy and laboratory examinations failed to prove the ante-mortem diagnosis. Then again, taking the weights of the organs into consideration, we may notice another fact. In our series, we took 54 cases of splenomegaly in which the weights of the organs varied from 600 grammes to 2,700 grammes. Out of these, 14 cases were diagnosed as hob-nail or kala-azar cirrhosis and in 2 there had been a suggestion of splenic anæmia. Out of the remaining 38 cases, no definite conclusion could be reached.

in 17, and in 21, chronic malaria or leishmaniasis had been suspected. Careful necropsy and histological examinations failed to show any evidence of either malaria or kala-azar in any one of them.

An attempt has been made to study the ætiology of these cases from the available history and the post-mortem records. While in the present state of our knowledge, it is not possible to find out the exact causation of these huge splenic enlargements, nevertheless we find many interesting facts which are well worth serious consideration. Clinically, there are always some features which are common to all

(1) They are always found in largest number to come from certain districts of Bengal, particularly West Bengal and the lower part of Eastern Bengal.

(2) They always run a very chronic course extending over years, carrying the enormous spleen and liver all the time and following their usual profession in life, though with much reduced efficiency.

(3) They always suffer from an irregular type of fever, the course of which does not follow that of any definite known malady. This fever is variously labelled by different medical men as malaria, leishmaniasis, enteric and the like.

(4) Futility of all therapeutic measures constitutes a very important factor. All the known remedies for splenic enlargements, such as quinine, iron, arsenic, antimony, etc., produced no impression on the course of the disease, some of the cases getting numerous injections of arsenic and antimony, on the supposition that the cases were chronic malaria or kala-azar.

(5) They always develop a secondary type of anæmia of varying grades depending on the intensity and duration of the disease, with marked diminution of white blood cells and polymorphonuclear leucocytes.

(6) They always tend to develop fibrosis of the liver, many of the cases ultimately changing into a typical picture of cirrhosis of the liver with ascitis.

(7) They always die of some intercurrent malady such as pneumonia, ulcerative colitis, cancrum oris, etc.

In the necropsy room, we find capsular changes in the way of thickening and perisplenitis and the development of adhesions to the surrounding structures specially to the diaphragm very frequent. The substance of the spleen feels firm and cuts with varying degrees of resistance, according to the duration of the illness. The cut surface always shows numerous prominent bluish white trabeculæ. The liver in these cases is always found to be enlarged and congested and, in some very old-standing cases, fibrotic and even hob-nailed. We systematically examined the bone-marrow in the middle third of the femur and in almost all of them we found the yellow marrow replaced by red formative marrow.

The histological examination of these spleens reveals a picture which is consistently found in every one of these cases, with minor modifications. In all of them, the splenic pattern tends to be lost and the malpighian follicles atrophied or

totally destroyed. The entire pulp looks cellular, congested and full of many large phagocytic mononuclear cells. In some of the cases, the latter show intense red cell phagocytosis indicating an abnormally increased capacity of the organ for blood destruction. The capsules are thickened and the trabeculae very prominent containing much fibrous tissue. There is also a considerable increase of reticular fibres which appear fairly coarse and numerous when stained with Foot-Bielschowsky's method.

Tuberculosis appears to be a fairly important cause of moderate degrees of splenomegaly. In our series of 525 cases of enlarged spleens, 88, i.e., about 6 per cent, were due to this cause. The enlargement was noticed particularly in chronic pulmonary infection with or without evidences of secondary tuberculous deposits. There had been thickening of the capsule and various degrees of toughness due to some increase of connective tissue in the trabeculae. Amyloid changes were found in none of the cases under the naked eye and only very rarely under the microscope.

The group of cases where great enlargement of the spleen was associated with typical cirrhosis of the liver deserves special attention. During the period of 11 years from 1920-1930, there have been 36 cases of cirrhosis of the liver. The picture was typical both before and after death and the liver in every case showed typical multilobular type of cirrhosis varified under the microscope. Out of this number, there were 21 cases in which the average weight of the spleen was found to be about 300 grammes and there were 15 cases in which the average rose up to 900 grammes. A large amount of capsular changes, considerable adhesions to the neighbouring structures and the generalized toughness of the substance of the organ characterized each one of them. In at least four of the last group, an ante-mortem diagnosis of kala-azar cirrhosis was made but no evidences of leishmaniasis could be detected by a careful and painstaking search both by direct smears and histological examinations of the spleen and liver after death.

Thus, we see that amongst our necropsy cases the typical portal type of cirrhosis may be classified into two distinct groups, one associated with marked splenomegaly and the other without it. Some of the cases in the former group have been considered to be due to the effect of leishmaniasis without any proof. From a very painstaking and critical survey of 25 parasite positive cases of kala-azar we definitely failed to find excessive production of fibrous tissue in the spleen and liver, nor any true portal cirrhosis of the liver (vide *Ind Jour Med Res*, 19, No 2, p 457). Besides, when the liver shrinks considerably in advanced cirrhosis and the radicles of the portal vein are compressed, there may be some enlargement of the spleen, mostly due to passive congestion of the organ and partly due to the activity of the reticulo-endothelial group of cells to cope with any chronic infection. The average weight of an organ in such a case is about 10 oz (Morley Fletcher). But certainly the above facts cannot account for the production of such huge spleens as we have met with in our present series of cases. Moreover had the conditions of

portal cirrhosis been responsible for such a picture, then such an enlargement of the spleen would be found in all cases without exception. Thus we are forced to conclude that these two groups of cirrhosis cases have two separate ætiologies. Whether this huge enlargement of the spleen is the cause, or the effect, of changes in the liver will be the subject-matter of a contribution to be published very shortly. Personally, I have ample reasons to believe that in these cases the changes in the spleen are primary and play the most important rôle in the ætiology of hepatic cirrhosis in Bengal.

My best thanks are due to Major G. Shanks, R M S, late Professor of Pathology, Medical College, Calcutta. The entire cost of the work has been borne by the Indian Research Fund Association to which I express my indebtedness.

THE PHARMACOLOGICAL ACTION OF THE VENOM OF RUSSELL'S VIPER OF INDIA (DABOIA OR *VIPERA ELEGANS*)

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[Received for publication, November 23, 1931]

In a previous paper embodying the results of the investigation of the venom of the Indian cobra, Chopra and Iswariah (1931) pointed out that the main action of the venom of the cobra is a stimulation of the respiratory and the vasomotor centres in the medulla, death being most probably due to over-stimulation of the respiratory centre leading to spasm of the muscles engaged in the movements of respiration. Death after the bite of the viper has for a long time been alleged to be due to capillary hæmorrhage or intra-vascular clotting. Most of the literature that deals with snake-bites classify snake venom into two broad divisions—the venom of the cobra and that of the viper. Apart from the difficulty of identifying the variety of snake, there has been a tendency to assign all cases of bite by a snake other than the cobra to the bite of the viper group, the venom of all the members of which is said to act on the system in an identical way.

Weir Mitchell and his collaborators (1886) working on the venom of poisonous serpents said that they found it difficult to secure the poison of *Daboia russelli* of India. Government aid and private enterprise alike failed to procure for them a sufficient quantity of the venom of this dreaded reptile. Having investigated partially the complex chemical nature of the venom, the above workers said that their discovery might be made the groundwork in India for a study of the poison of the Daboia. But so far it has not been possible to get at any work calculated to throw more light on the action of the venom of the Russell's viper.

The Daboia or the Russell's viper is responsible next to the cobra for the greatest number of deaths from snake-bite. It is a viper according to zoological classification. The action is also said to resemble that of the other vipers. But the two main actions ascribed to the venom—intravascular clotting and hæmorrhage—did not appear to us to be the cause of the high mortality from the bite of the Russell's viper. Hence this investigation. The venom, we investigated, was supplied to us by the Haffkine Institute, Bombay, in the form of orange crystals fairly soluble in water. During the course of our work it was kept in cold storage in order to prevent deterioration.

Chemistry

Edward T. Reichart and Weir Mitchell (1886) in two examinations separated from the viper venom two bodies corresponding to those in the cobra venom—'a water-venom-globulin' and a peptone. The water-venom-globulin exists only in an exceedingly small quantity. The peptone which is more abundant dialyses with greater difficulty than that of the cobra venom. The venom of the Daboia is not so resistant to heat as that of the cobra. The vigorous frothing on shaking suggests the presence of saponin, but other chemical tests do not seem to indicate its presence. The precipitation and partial detoxication on heating indicates to some extent that the toxic factor is of the nature of globulin either alone or with others in combination. Its toxicity is easily destroyed by caustic agents.

Toxicity

Calmette (1908) in his book on venoms gives the M. L. D. of the Daboia venom as 0.001 gm. for guinea-pigs weighing on an average 600 gm. or roughly 2 mg. per kilo. Lamb, quoted by the above, obtained 0.05 mg. per kilo for rabbits by the intravenous route. Our work with white rats, guinea-pigs, cats and dogs indicated 2 mg. per kilo roughly as the lethal dose thus confirming Calmette's findings. Guinea-pigs were a little more susceptible than white rats, and dogs more than cats.

Very often there were convulsive spasms of the muscles of the limb before death. Anæsthetized animals also showed these spasms sometimes. The spasms invariably started when the blood-pressure was at its lowest. Fayrer and Wall (1886) had observed the same and Weir Mitchell *et al.* noticed this even after artificial respiration. The coincidence of the fall of blood-pressure with the onset of the convulsive spasms seems to indicate that deficient blood supply to the higher nerve centres is the cause for this.

Post-mortem appearances

The following were noticed. Slight congestion of the lungs with a few patches of hæmorrhage here and there, right heart semi-distended with livid blood,

no clots noticed in the chambers of the heart or the larger blood vessels. The alimentary tract was cyanosed with distended veins, the spleen and the liver often showed no change. The kidneys were livid with prominent distended veins. Blood-tinged effusions were often seen in the pleural and the peritoneal cavities.

Local action

The bite of a viper has been noticed to produce an intense local irritant effect, necrosis invariably sets in around the site of the bite. Manson-Bahr, in his book on 'Tropical Diseases', observed that viper venom caused severe pain locally with rapidly forming extensive cedema, together with blood-stained discharge and ecchymosis round the site of the puncture. We injected 1 c.c. of a 1 to 5 per cent solution of the Russell's viper venom into the thigh muscles of white rats and guinea-pigs after removing the hair and securing aseptic conditions. If the animals did not die in 3 to 4 hours a slight reddening and swelling of the part were noticed. Controls with normal saline did not show any such response. No hardening was noticed at the site even after three days. Higher concentrations of the venom, i.e., 10 per cent or more in bigger animals, showed diffuse necrosis after 24 hours if the animal did not die in that period.

Action on blood

The venom does not hasten coagulation of blood *in vitro*. The hæmolytic property of the venom was tested in various concentrations on the erythrocytes of dog and man. In concentrations of 1 in 1,000 or more the red blood corpuscle hæmolyzed in 5 to 15 minutes. In higher dilutions no hæmolysis was noticed even in 2 to 4 hours. This was compared with the hæmolytic power of saponin and it was found that in dilution of 1 in 100,000 saponin hæmolyzed the red corpuscles in 1 to 2 minutes.

Circulatory system

Cats weighing roughly 2 kilos were mainly used for the systematic investigation and sometimes dogs of about 4 kilo body weight. Cats were anaesthetized with chloralose administered intramuscularly in doses of 0.1 gm. per kilo, and dogs with paraldehyde 2 c.c. per kilo given by mouth. The venom was prepared in saline, usually in 1 per cent concentration. 0.2 to 0.4 mg. of the venom injected with 2 c.c. of saline into the femoral vein of cats produced a gradual fall in the blood-pressure of about 20 to 35 mm. (Graph 1, fig. 2), the initial pressure being about 60 mm. This fall was fairly persistent. In the dog the fall of blood-pressure was much more sudden (Graph 1, fig. 1). A second dose of the same quantity did not elicit the same response, very often there was no response at all. If the animal survived two injections, subsequent administration even to the extent of

5 to 10 mg (3 to 5 times the lethal dose) did not act on the animal adversely. Very often when a big fall of blood-pressure occurred causing almost stoppage of heart action with the first or second dose, administration of about 10 c.c. of normal saline intravenously was able to revive the heart and bring the blood-pressure back nearly to the original level (Graph 1, fig 3, c).

Injections of the venom after paralysis of the sympathetics by ergotoxin showed the usual fall of blood-pressure in spite of its already low level due to vasoconstrictor paralysis (Graph 2, fig 1).

Histamine was administered in doses of 0.1 mg. to fresh animals so as to cause a permanent fall in the blood pressure. Injections of the venom in doses of 0.5 to 5 mg. after this did not produce a further fall of blood pressure (Graph 2, fig 3, a and b). If the process were reversed and histamine given after one or two injections of the venom a sustained rise of blood pressure was noticed in the place of the usual fall.

We next observed the action of the venom on the volumes of the splanchnic organs. These showed a fall only in correspondence with the general fall of the blood-pressure. The volume of the limb showed no change nor was any change noticed on perfusing the mesenteric vessels. But perfusion of the limb *in situ* showed a definite quickening of the inflow as well as the outflow.

The action of the venom on the heart *in situ* was recorded by the myocardiograph and the cardiometer. In both it was found that the heart was not appreciably affected, but the slight changes sometimes noticed seemed to be consequent on the fall in blood-pressure (Graph 1, fig 2). The isolated heart of the rabbit, the kitten as well as that of the frog *in situ* was unaffected by the venom in dilutions of 1 in 500,000 to 1 in 20,000 (Graph 1, fig 3, a).

Respiratory system

Record of intra-tracheal respiration in anaesthetized cats did not show any change after administration of viper venom (Graph 2, fig 2). The venom had no action on the lower respiratory mechanism as shown by experiments recording intrapleural pressure. In dogs, sometimes, the first dose of the venom caused arrest of respiration after a marked fall in the blood-pressure. In such cases the animal never revived.

DISCUSSION

Death after the bite of the Russell's viper has been so far attributed to intravascular clotting or multiple hæmorrhages. Carmichael Low, in Price's Medicine, claims for the viper venom a paralytic action on the vasomotor centre. But our experiments lead us to the conclusion that the general fall in the blood-pressure

noticed after the administration of the viper venom is due to the poison acting on the circulation peripheral to the arterioles

That the fall in the blood-pressure is not mainly due to the action of the venom on the heart is shown by the isolated heart being unaffected by concentrations even higher than what is obtained in the body in fatal cases. *In situ* the ventricle is augmented progressively as the blood-pressure falls. The splanchnic organs show a fall in volume corresponding to the fall in blood-pressure. This indicates that the fall in the blood-pressure is not due to active dilatation of the arterioles of the splanchnic region. The general fall in the blood-pressure is noticed even after paralysing the vaso-constrictors with ergotoxin. This again shows that the vasomotor system is not primarily responsible for the fall in the blood-pressure. The lowering of the blood-pressure caused by the venom after ergotoxin is more abrupt than in normal animals. This indicates that before ergotoxin was administered the tone of the arterioles in virtue of the intact vasomotor nerve endings in them permitted only a gradual fall of blood-pressure, but that when this tone was lost after the injection of ergotoxin the fall was more abrupt. That the venom does not further lower the blood-pressure after repeated injections of histamine shows that it most probably acts similarly, namely, as a paralysing agent of the capillaries. It has also been noticed that histamine administered after the venom causes a prolonged rise of blood-pressure. This fact seems to indicate that the capillaries have already been completely paralysed by the venom and cannot be further dilated by histamine, but that the histamine action on the arterioles is responsible for the sustained rise in the blood-pressure.

The capillary action is also indicated by the acceleration of the flow of the perfused fluid through the limb after the injection of viper venom. This effect is not noticed when the mesenteric vessels are perfused, probably due to the meagre distribution of capillaries in the splanchnic area unlike the limb and the surface. That a failing heart due to general fall of blood-pressure could be revived with 10 to 15 c c of saline, in our experimental animals, lends support to the hypothesis that as a result of the administration of viper venom the blood stagnates in the capillaries and the heart fills less effectively. This suggests the possible line of treatment in cases of poisoning by viper venom.

Our investigation also shows that once the heart adapts itself to the maximum capillary paralysis and the consequent fall of blood-pressure, the animal could tolerate even 5 to 10 times the lethal dose of the venom.

SUMMARY AND CONCLUSIONS

- 1 The venom of the Russell's viper of India causes a general fall of blood-pressure in mammals, probably due to the paralysis of the capillaries.
- 2 Even in high concentrations, the venom does not seem to possess any direct action on the heart, nor has it any action on the walls of the blood vessels.

3 Intravenous administration of normal saline in about 10 to 15 c c doses tend to revive the animal, the heart works for a fairly long time after the saline administration with a good rate and amplitude

4 There is no evidence that the venom of the Russell's viper increases the coagulability of blood, nor that hæmolysis is caused by such concentration as is likely to be found in the human system after a bite by a viper

We are greatly indebted to Lieut -Colonel R N Chopra, I M S, of the Calcutta School of Tropical Medicine, for having suggested this work and for kindly supplying the venom. We also thank Dr K Venkatachalam, Research Officer in Indigenous Drugs at this institution, for his constant help throughout the course of this research

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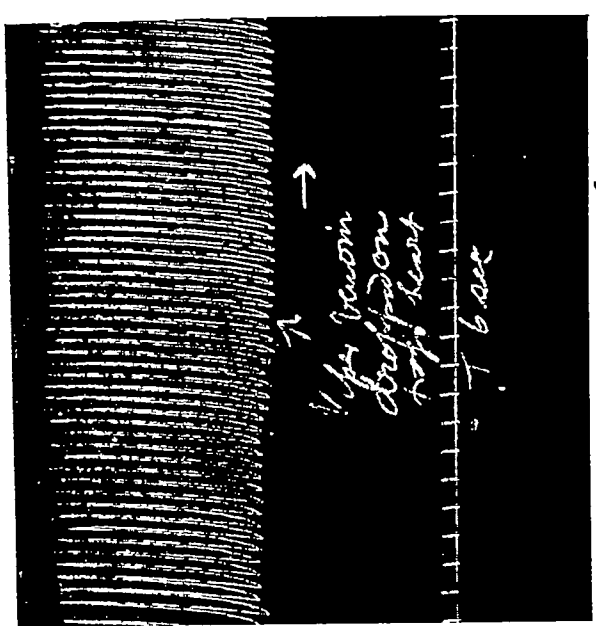
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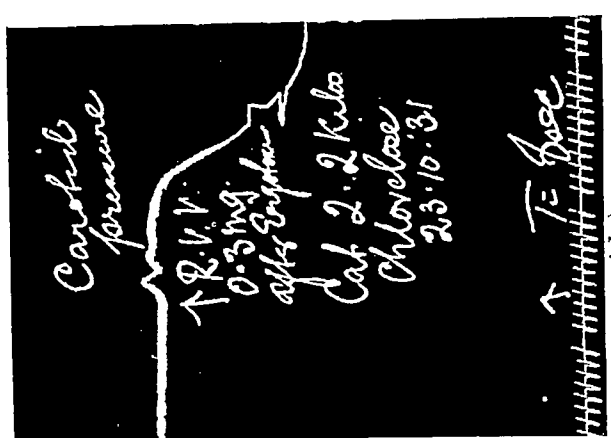
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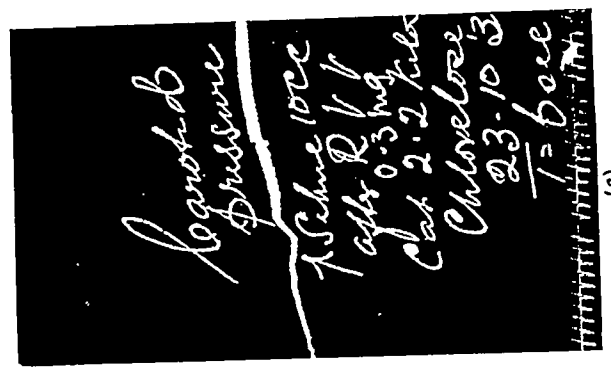
Fig 2 --Urethane cat--myocardiograph and blood-pressure Note the gradual augmentation



(a)



(b)



(c)

Fig 3 (a) The venom has no action on the heart of a frog (b) Note the abrupt fall in the blood pressure after ergonov. pro-

1 --Paraldehyde dog--the venom causes a sudden fall in the blood-

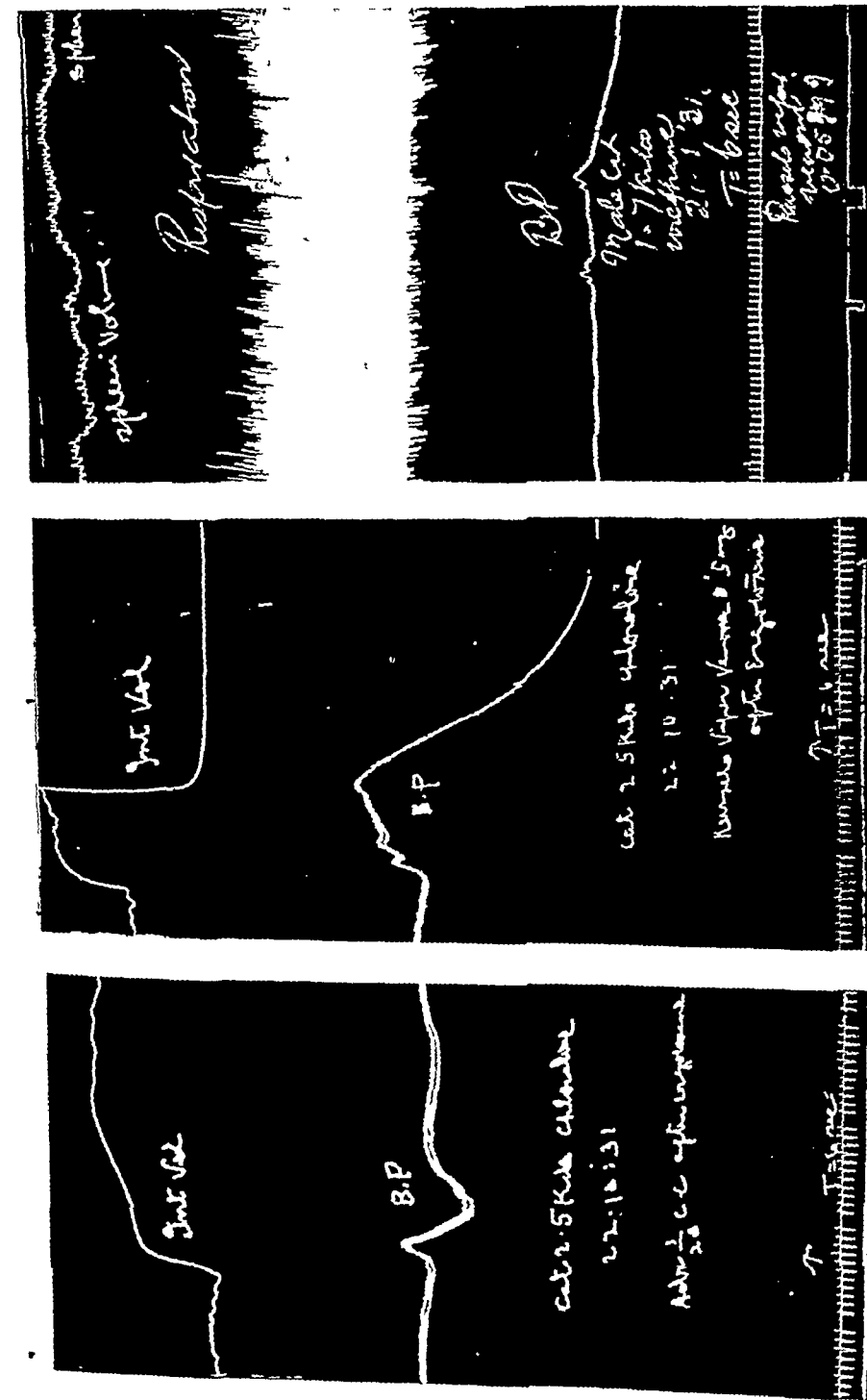


Fig 2 — Russell's viper venom shows no action on the respiration or on the volume of the spleen

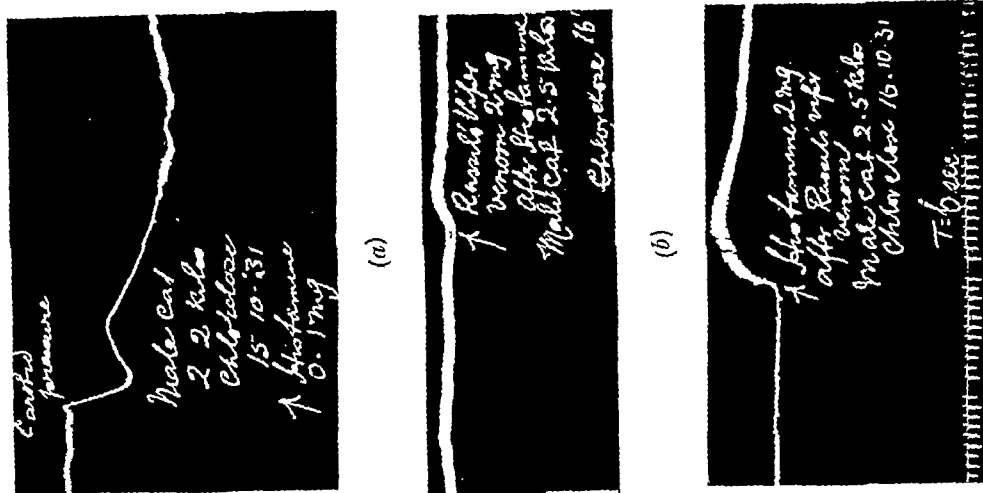


Fig 3 — Chloralose cat recording blood-pressure

- (a) Histamine causes a fall in the blood-pressure
- (b) The venom has no action after histamine
- (c) Histamine after the venom gives a prolonged rise

Fig 1 — Chloralose cat showing carotid blood pressure and volume of the intestine
Note the fall in the blood pressure and rise in the intestinal volume by adrenalin administered after the sympathetics have been paralysed by ergotoxin. The venom after this showed the usual fall in the blood pressure with the consequent fall in the intestinal volume

STUDIES IN THE NUTRITIVE VALUE OF INDIAN VEGETABLE FOOD-STUFFS.

Part IV.

NUTRITIVE VALUE OF GREEN GRAM, *PHASEOLUS MUNGO* AND BLACK GRAM, *PHASEOLUS RADIATUS*

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[Received for publication, November 23, 1931]

THE *Phaseolus* is a genus of climbing plants, usually herbaceous, of which some fifteen species are indigenous to India. The aconite bean—*Phaseolus aconitifolius*—has already been referred to in the previous part.

The green gram—*Phaseolus mungo*—is grown in all parts of the country. In the Bombay Presidency it occupies an area of two and a half lakhs of acres. It is chiefly cultivated in the Kharif season when it is grown subordinate to jwar and other cereals. It is also sown alone as a *rabi* crop in some districts as a second crop after rice. The pulse is eaten boiled whole, or is split and used as dal. There are three varieties of the pulse—green, black and yellow. The green, being the most common, was employed for purposes of this investigation.

The black gram—*Phaseolus radiatus*—is an important crop in the black soils of Khandesh. Nearly three lakhs of acres are under the cultivation of this crop. The ripe black grain is very much esteemed as a pulse. It is the chief constituent of the wafer biscuits known as *papads*. Both the grain and straw are valuable

horse and cattle food There are two varieties of the pulse one with large black seeds and the other with small green seeds, of which the former was used for this work

The above two pulses are known by the following vernacular names —

Green gram (*Phaseolus mungo*) mung, patcha payaru, uruthulu, etc

Black gram (*Phaseolus radiatus*) urud, samra, mashkulai, udid, uddu, etc

The flour of these two pulses dried at 100°C gave the following percentages on analysis —

TABLE I

	Ash	Ether extractives	Crude fibre	Crude protein N × 6.25	Carbohydrate by difference	True protein
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
<i>Phaseolus mungo</i>	4.14	2.31	5.59	27.94	59.71	25.25
<i>Phaseolus radiatus</i>	4.58	2.40	4.31	28.32	62.38	25.00

The globulins of these two pulses were isolated and an analysis made of them by methods similar to those described in the previous parts. The globulin preparations were cream coloured and gave all the colour tests for proteins and contained sulphur, tyrosine and tryptophane. The results of their elementary analysis, Van Slyke analysis and the estimation of the essential amino acids in them are given in Tables II, III and IV respectively —

TABLE II

	<i>Phaseolus mungo</i>		<i>Phaseolus radiatus</i>	
Moisture	8.85	8.63	9.74	9.60
Ash	0.45	0.54	0.48	0.51
	Ash and moisture free			
Nitrogen	15.68	15.39	13.82	13.64
Sulphur	0.52	0.48	0.99	0.97

TABLE III

Analysis of the globulin preparations by the Van Slyke method (expressed as per cent of total nitrogen)

Form of nitrogen	GLOBULIN OF			
	<i>Phaseolus radiatus</i>		<i>Phaseolus mungo</i>	
Acid insoluble melanin	2 38	2 16	1 15	1 24
Acid soluble melanin (adsorbed by lime)	0 43	0 71	0 90	0 86
Amide .	11 11	11 06	11 12	11 51
Diamino —				
Arginine	14 85	14 92	15 90	16 12
Histidine	7 14	6 47	2 48	2 62
Cystine	1 05	0 88	0 39	0 41
Lysine	8 12	9 03	9 95	9 17
Mono amino —				
Amino	55 00	53 36	53 62	52 18
Non amino	2 16	2 62	5 06	5 67
TOTAL	102 27	101 21	100 87	99 78
By direct estimation	4 37	4 44	4 86	4 99
Half lysine nitrogen	4 06	4 52	4 98	4 59

TABLE IV

Expressed as per cent of protein (ash- and moisture-free)

Amino acid	GLOBULIN OF		Method
	<i>Phaseolus radiatus</i>	<i>Phaseolus mungo</i>	
Lysine	6.16	7.70	} Van Slyke
Histidine	3.16	1.16	
Arginine	6.37	7.90	
	7.17	8.80	Plimmer and Rosedale (1925)
Cystine	3.51	1.59	Remington (1930)
Tyrosine	3.63	3.35	Fohn and Merenzie (1929)
	3.87	3.92	Zuwerkalao (1926)
Tryptophane	0.80	0.59	Tillman and Alt (1925)
	0.81	0.71	Fohn and Merenzie (1929)

The above tables show that the globulin of *Phaseolus radiatus* has a low nitrogen content, but the percentage of sulphur in it is high and consequently its cystine content also

For determining the biological values, the metabolic experiments were carried out at a 10 per cent level of protein intake. The modified type of Coonoor cage described in the previous paper was employed. In Part I of this series, the biological values of the proteins of pigeon pea—*Cajanus indicus*—and field pea—*Pisum arvense*—were determined at a five per cent level of intake. As these values in the case of the other pulses were worked out at a ten per cent level the metabolic experiments with these pulses were repeated at the higher level for purposes of comparison. In preparing the pulse protein rations, the flour of the decorticated seed was employed in all cases except that of *Pisum arvense* which is eaten whole. The metabolism data are given in Tables V and VI. The available or net protein values of these pulses calculated from their digestibility and biological values are given in Table VII.

TABLE V

Rat number	Initial weight		Food intake		Nitrogen intake	Faecal nitrogen	Urinary nitrogen	Metabolic nitrogen in faeces per gramme of food	Endogenous nitrogen in urine per 100 g body-weight	Food nitrogen in faeces	Absorbed nitrogen	Food nitrogen in urine	Total nitrogen retained	Digestibility	Biological value
	g	g	g	g	mg	mg	mg	mg	mg	mg	mg	mg	mg	Per cent	Per cent
<i>Period 1 protein free ration (N = 0.075 per cent)</i>															
31	63.0	56.0	4.25	3.17	13.87	17.73	3.263	26.67							
32	61.1	57.7	4.19	3.13	19.31	16.02	4.608	26.97							
33	66.0	60.9	5.10	3.81	16.30	18.88	3.197	29.73							
34	77.0	71.0	4.51	3.39	11.64	21.59	2.564	29.18							
35	77.0	73.0	5.04	3.76	17.73	20.87	3.519	27.84							
36	82.1	76.9	4.69	3.50	13.16	20.59	2.803	25.90							
<i>Period 2 Pisum arvense flour ration (N = 1.636 per cent)</i>															
31	60.5	67.0	5.91	96.70	40.57	42.29				22.67	74.03	25.02	40.01	77	66
32	60.0	62.1	4.93	96.50	48.21	35.79				28.24	58.26	20.01	38.25	67	66
33	61.5	67.2	5.93	97.03	49.55	45.98				31.15	66.58	27.07	39.51	69	59
34	75.1	81.0	5.89	96.36	44.19	45.78				29.45	66.91	23.88	43.03	69	64
35	75.0	79.9	6.24	102.10	52.56	51.25				30.52	71.58	29.75	41.83	70	58
36	80.6	87.0	6.03	98.65	48.18	49.18				31.82	66.83	26.10	39.43	68	59
												Average		70	62

TABLE V—concd

Rat number	Initial weight		Food intake	Nitrogen intake	mg	Faecal nitrogen	mg	Urine nitrogen	mg	Metabolic nitrogen in faeces per gramme of food	mg	Endogenous nitrogen in urine per 100 g body-weight	mg	Food nitrogen in faeces	mg	Absorbed nitrogen	mg	Food nitrogen urine	mg	Total nitrogen retained	Per cent	Digestibility	Per cent	Biological value
	g	g																						
Period 3 Phaseolus radiatus flour ration (N = 1.814 per cent)																								
31	74.0	77.0	5.52	100.10	34.51	49.47			19.08	81.02	28.72	52.30	81	65										
32	71.8	80.3	6.44	116.80	39.84	54.07			17.24	99.56	36.19	63.37	85	64										
33	75.4	86.2	7.82	141.91	52.36	60.81			29.61	112.30	44.71	67.59	79	60										
34	91.8	101.3	7.02	127.40	40.23	60.03			23.09	104.31	34.05	70.25	82	67										
35	89.0	95.0	7.06	128.00	47.39	63.56			22.48	103.52	38.42	67.08	82	63										
36	93.2	97.7	7.16	129.90	42.40	61.84			22.92	106.92	36.93	69.97	82	65										
													Average	82.0	64									
Period 4 Protein free ration (N = 0.075 per cent)																								
31	71.0	63.2	4.46	3.35	11.50	18.72	2.561	27.39																
32	75.0	64.8	3.55	2.65	10.42	16.44	2.938	23.53																
33	80.1	69.3	4.40	3.29	12.16	21.39	2.765	28.64																
34	90.2	82.2	4.40	3.29	10.48	22.19	2.382	25.74																
35	85.1	77.0	4.62	3.45	15.44	22.46	3.559	27.52																
36	94.3	85.5	4.06	3.03	10.88	23.53	2.680	26.17																

TABLE VI
Metabolism data

metabolism

Rat number	Initial weight		Final weight		Food intake		Nitrogen intake		Fæcal nitrogen		Urinary nitrogen		Metabolic nitrogen in faeces per gramme of food		Endogenous nitrogen in urine per 100 g body weight		Food nitrogen in faeces		Absorbed nitrogen		Food nitrogen in urine		Total nitrogen retained		Digestibility		Biological value	
	g	g	g	g	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	Per cent	Per cent	Per cent	Per cent
Period 1 Protein free ration (N = 0.075 per cent)																												
25	63.0	60.0	3.92	2.93	11.64	15.02	2.970	24.97																				
26	70.8	65.1	3.97	2.96	9.78	20.36	2.463	29.99																				
27	65.5	59.7	4.11	3.07	13.87	20.79	3.534	32.21																				
28	73.0	66.0	3.83	2.86	11.58	17.02	3.025	24.48																				
29	83.1	78.5	4.55	3.40	14.97	19.59	3.269	24.25																				
30	64.9	60.5	4.54	3.39	14.73	19.73	3.244	31.47																				
Period 2 Cajanus indicus flour ration (N = 1.50 per cent)																												
25	62.0	65.2	4.92	73.91	38.32	29.74												24.70	49.11			14.30	34.81		67		71	
26	69.0	79.8	6.55	98.24	43.33	39.23												23.97	74.27			17.32	56.75		77		76	
27	62.0	72.0	6.39	95.85	52.60	34.88												29.87	65.98			14.09	51.89		69		79	
28	64.8	75.0	6.51	97.66	53.88	33.37												33.54	64.12			15.97	48.15		67		75	
29	80.0	84.8	6.12	91.81	45.32	41.08												25.44	66.37			20.29	46.08		72		69	
30	63.1	68.5	5.43	81.45	38.19	38.03												20.93	60.52			17.10	43.42		74		72	
																							Average		71		74	

TABLE VI—*concl'd*

Rat number	Initial weight		Final weight		Food intake		Nitrogen intake		Fecal nitrogen		Urinary nitrogen		Metabolic nitrogen in faeces per gramme of food		Endogenous nitrogen in urine per 100 g body weight		Food nitrogen in faeces		Absorbed nitrogen		Food nitrogen in urine		Total nitrogen retained		Digestibility		Biological value				
	g	g	g	g	g	g	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	per cent	per cent	per cent	per cent			
Period 3 Phased plus mungo flour ration (N = 1.808 per cent)																															
25	69.5	75.2	5.38	102.70	33.49	48.73											19.67	83.03	31.67	51.36					81	62					
26	86.2	88.1	6.82	130.10	41.63	68.79											18.14	111.96	43.95	68.05					85	61					
27	82.3	89.2	7.35	140.30	44.27	72.82											17.96	122.34	48.08	74.22					87	61					
28	83.1	92.0	7.41	141.40	46.47	68.80											22.58	118.82	44.65	74.15					84	62					
29	95.5	103.0	8.19	156.30	50.81	81.50											24.39	131.91	55.48	76.42					84	58					
30	76.5	81.2	7.20	137.40	59.37	68.07											32.14	105.26	45.15	60.15					77	57					
																Average															
																												93		60	
Period 4 Protein free ration (N = 0.075 per cent)																															
25	70.5	65.0	3.78	2.82	8.96	15.67																									
26	82.0	69.7	3.32	2.48	13.10	20.90																									
27	82.2	71.3	4.12	3.08	14.84	20.46																									
28	83.0	73.1	3.54	2.64	11.77	20.05																									
29	96.3	83.0	4.59	3.43	14.71	24.39																									
30	75.0	66.2	4.25	3.17	12.96	19.65																									
																Average															
																												22.88			
																												27.55			
																												26.63			
																												25.67			
																												27.18			
																												27.84			

TABLE VII

Pulses	Total protein $N \times 6.25$	Net protein value
<i>Cajanus indicus</i>	25.63	13.47
<i>Pisum arvense</i>	25.57	11.01
<i>Phaseolus mungo</i>	27.94	13.92
<i>Phaseolus radiatus</i>	26.32	13.81

So far ten of the most common Indian pulses have been investigated and it will be interesting at this stage to compare the results of these investigations. The comparative statements are given in Tables VIII, IX and X. Tables VIII and IX give respectively the Van Slyke analysis and amino acid contents of the ten globulins. In carrying out the Van Slyke analysis, the methods and conditions detailed out in Part I of the series, were strictly adhered to in all the ten cases, so that the results may be comparable. In Table X are given the results of the metabolic experiments.

A study of the above tables reveals the following facts —

Van Slyke and Birchard (1911) have suggested that one of the amino groups of lysine is free and furnishes a large part of the free amino nitrogen in a protein. This seems to hold good in the case of all the ten globulins investigated as in each case the free amino nitrogen very nearly corresponds to half its lysine nitrogen content.

The non-basic non-amino nitrogen of *Cicer arietinum* globulin is very low. It may perhaps be due to its poor content of proline all of whose nitrogen is in the non-amino form.

Cajanus indicus globulin seems to possess less basic nitrogen than the others. This is accounted for by its low arginine content. *Cicer arietinum* and *Lens esculenta* globulins are the richest in their arginine content being very nearly double that of *Cajanus indicus*. As a consequence these two globulins contain higher amounts of basic nitrogen.

Phaseolus radiatus globulin contains 3.51 per cent cystine which is much higher than the average cystine content of the other globulins which ranges between 1.24 per cent (*Dolichos biflorus*) and 2.02 per cent (*Cicer arietinum*).

TABLE VIII

Comparative statement of the results of the Van Slyke analyses of the globulins of the following ten legumes expressed as percentages of total nitrogen

Form of nitrogen	<i>Phaseolus acutifolius</i>	<i>Dolichos biflorus</i>	<i>Dolichos lablab</i>	<i>Vigna calyans</i>	<i>Pisum arvense</i>	<i>Lens esculenta</i>	<i>Cajanus indicus</i>	<i>Phaseolus mungo</i>	<i>Phaseolus radiatus</i>	<i>Cicer arietinum</i>
Melanin	1.70	1.30	0.84	1.75	2.29	1.13	1.36	2.80	2.84	1.03
Amide	12.02	11.27	8.55	11.13	10.60	10.21	10.13	11.47	11.10	10.53
Diamino —										
Arginine	15.73	12.41	16.91	15.23	18.59	20.40	11.93	16.01	14.89	20.22
Histidine	5.34	5.21	2.39	3.80	3.12	4.79	4.41	2.55	6.81	2.29
Cystine	0.40	0.65	0.71	0.52	0.22	0.50	0.46	0.40	0.97	0.94
Lysine	6.59	10.14	8.99	9.14	9.55	7.86	8.56	9.56	8.58	8.79
Mono amino —										
Amino	55.63	59.52	56.79	54.28	50.59	53.82	61.41	52.90	54.18	56.47
Non amino	3.18	1.62	3.77	4.12	4.90	2.36	2.45	5.37	2.39	0.77
TOTAL	100.59	102.12	98.93	99.97	99.92	100.87	100.71	101.06	101.76	101.04
By direct estimation	3.61	4.94	4.46	4.92	4.58	4.11	5.11	4.93	4.39	4.71
Half lysine nitrogen	3.29	5.07	4.50	4.57	4.78	3.93	4.28	4.79	4.29	4.35

Free amino nitrogen in the native protein

TABLE IX.

Comparative statement of the contents of the essential amino acids in the globulins of the following ten pulses
(expressed as percentage of protein, ash- and moisture-free)

Name of amino acid	<i>Phaseolus acutifolius</i>	<i>Dolichos biflorus</i>	<i>Dolichos lablab</i>	<i>Vigna catjang</i>	<i>Pisum arvense</i>	<i>Lens esculenta</i>	<i>Cajanus indicus</i>	<i>Phaseolus mungo</i>	<i>Phaseolus radiatus</i>	<i>Cicer arietinum</i>	Method
Lysine	5.49	8.15	7.27	5.96	8.19	6.70	7.03	7.70	6.16	7.42	Van Slyke
Histidine	3.15	2.38	1.38	2.21	1.90	2.77	2.56	1.46	3.46	1.42	
Arginine	7.81	6.00	8.09	7.45	9.50	10.35	5.84	7.90	6.37	10.29	
Cystine	8.62	7.07	8.72	7.91	10.64	11.53	6.91	8.80	7.17	11.85	Plimmer and Rosedale (1925)
Tyrosine	1.62	1.24	1.54	1.89	1.99	1.62	1.86	1.59	3.51	2.02	Remington (1930)
Tryptophane	3.49	4.01	4.34	3.74	3.74	3.56	3.16	3.35	3.63	2.95	Fohn and Marenzie (1929)
	3.76	5.74	5.11	3.88	1.98	3.92	3.12	3.92	3.87	4.90	Zuwerkalao (1926)
	0.67	0.76	0.44	0.80	0.54	0.71	0.41	0.59	0.80	0.46	Tillman and Alt (1925)
	0.74	1.02	0.42	0.59	0.51	0.62	0.46	0.74	0.84	0.46	Fohn and Marenzie (1929)

TABLE X

Pulses arranged in the ascending order according to

Digestibility of the protein		Biological value of the protein		Net protein value of the pulse	
	Per cent		Per cent		Per cent
<i>Vigna catjang</i>	58	<i>Dolichos lablab</i>	57	<i>Phaseolus aconitifolius</i>	9.04
<i>Phaseolus aconitifolius</i>	59	<i>Phaseolus aconitifolius</i>	57	<i>Dolichos biflorus</i>	10.43
<i>Dolichos biflorus</i>	59	<i>Lens esculenta</i>	58	<i>Dolichos lablab</i>	10.65
<i>Dolichos lablab</i>	65	<i>Phaseolus mungo</i>	60	<i>Vigna catjang</i>	10.86
<i>Pisum arvense</i>	70	<i>Pisum arvense</i>	62	<i>Pisum arvense</i>	11.10
<i>Cajanus indicus</i>	71	<i>Phaseolus radiatus</i>	64	<i>Lens esculenta</i>	12.86
<i>Cicer arietinum</i>	76	<i>Dolichos biflorus</i>	67	<i>Cajanus indicus</i>	13.47
<i>Lens esculenta</i>	78	<i>Vigna catjang</i>	72	<i>Phaseolus radiatus</i>	13.81
<i>Phaseolus radiatus</i>	82	<i>Cajanus indicus</i>	74	<i>Phaseolus mungo</i>	13.92
<i>Phaseolus mungo</i>	83	<i>Cicer arietinum</i>	78	<i>Cicer arietinum</i>	16.68

Note—Except in the cases of *Vigna catjang*, *Phaseolus aconitifolius*, *Dolichos biflorus* and *Pisum arvense*, the flour of the decorticated seeds were used for the feeding experiments. In the other cases the seed coats were not removed as they are generally eaten as such.

In estimating tyrosine by the method of Zuwerkalao much difficulty was experienced during colour comparison owing to the formation of slight quantity of precipitates in the unknown solution. If the precipitate is filtered off the tyrosine value is lowered as some colour is invariably absorbed by the precipitate. If it is retained, the value is enhanced by the cloudiness produced. Similarly in the estimation of tryptophane, it was found difficult exactly to match the pale yellow colours obtained in the method of Tillman and Alt. So in both cases it is preferable to take the values obtained by the method of Folin and Marenzeller to be the more trustworthy. The globulins of the pulses of the *Phaseolus* genus seem to be richer in their tryptophane content. *Dolichos biflorus*, however, is an exception, its tryptophane value being more than that of the other globulins.

In Table X the pulses are arranged in the ascending order according to biological values and digestibilities of their proteins (columns 1 and 2). Column 3 gives the net available protein content in each pulse. These values were obtained with diets containing approximately 10 per cent of the protein from the several pulses. *Cicer arietinum* proteins which are considered to be very nutritious possess

the highest biological value. It might be noted here that this legume is the most widely cultivated of all the Indian pulses.

Among the pulses belonging to the *Phaseolus* genus, the proteins of *Phaseolus acutifolius* are poor both in their digestibility, as well as in their biological value. Hence it occupies the lowest place when the pulses are arranged according to their net available protein contents. The proteins of the other two pulses of this genus are much better, their digestibility being even higher than that of *Cicer arietinum*.

The total protein content of pulses in general is about 25 to 28 per cent. The available protein, on the other hand, is much less. On the average only about 50 per cent of the total proteins in pulses are available for the building up and repair of tissues in the body.

It is of fundamental importance at this stage that we should recognize the limitations of the experimental methods we have employed for finding out the biological values. The nutritive value of a protein depends not only on its amino-acid make up, but also on the unknown biological factors that determine its utilization in the process of repair and growth. When a young animal is fed on a diet deficient in any respect, it makes use of the food as best as it can under the circumstances. Animals differ greatly in the degree of vitality with which they are born, and this is a variable factor which, to a certain extent, influences their ability to utilize the food faultily in any respect. In this way we can account for the variation in the biological values obtained with two different groups of rats or even with a group of rats belonging to the same litter.

In the second place, the biological values obtained as a result of feeding an animal with different pulse grains do not necessarily represent the nutritive value of the proteins only since the seeds contain non-protein nitrogenous substances as well. The latter are not probably alike in quality and quantity in any two pulse grains and it has not been possible either to isolate from any seed a preparation which contains its total proteins in the proportion in which these naturally occur in the grain, or to determine exactly the amount of protein consumed by the experimental animals when fed on the entire grains.

Thirdly, the crude fibre content of the pulses vary and form another factor influencing the results. Mitchell and Carman (1926) have shown that the faecal nitrogen increases with the amount of crude fibre eaten. For minimizing this error, it is proposed, in future, to keep the crude fibre content of the protein and non-protein rations as nearly the same as possible.

Finally, there was noticeable, in many cases, a distinct loss of appetite in the animals experimented upon, in spite of the small quantity of dried yeast given them. This was more pronounced during the periods of non-protein feeding and was not wholly absent during the periods of protein feeding. This state of things can be remedied by increasing the quantity of yeast in the diet. But, it would result in introducing a large quantity of nitrogen whose effect on the biological value is

unknown and which is not taken into account in calculating the metabolism data. Attempts are being made to get over this difficulty by using vitamin concentrates from yeast instead of whole yeast.

SUMMARY

The globulins of two Indian pulses—*Phaseolus radiatus* and *Phaseolus mungo*—have been isolated and analysed.

The biological values and digestibilities of the proteins of *Phaseolus radiatus*, *Phaseolus mungo*, *Cajanus indicus* and *Pisum arvense* have been determined by Mitchell's method at a 10 per cent level of intake. From these values the net available protein contents of the pulses have been calculated.

Comparative statements are given of the analyses of the globulins of ten of the most common Indian pulses and also of the results of the metabolic experiments with these pulses. It is found, on an average, that only about 50 per cent of the total proteins in these pulses are available for the processes of growth and repair in the animal body.

The possible sources of error in such experiments are discussed and remedies suggested.

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PROTEIN GRAPHS IN SYPHILIS WITH THEIR RELATION TO THE WASSERMANN REACTION

BY

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(Research financed by a grant from the Indian Research Fund Association)

[Received for publication, November 23, 1931]

INTRODUCTORY

HAVING investigated in a somewhat detailed manner the serum protein changes which occur in kala-azar and having shown by graphs the way in which these react to specific antimony treatment (Lloyd and Paul, 1928, Lloyd, Napier and Paul, 1929) we naturally turned to the case of syphilis in which disease protein changes occur which are believed to be the basis of some of the simpler tests used in its diagnosis, e g , Nonne's test

The graphs included in the first part of this paper were all taken from definitely diagnosed cases of active generalized syphilis in the 'secondary' stage. The patients were all females in the Voluntary Lock Hospital, Calcutta. All showed typical clinical signs of secondary syphilis and a positive Wassermann reaction. Several of them were also suffering from gonorrhœa which we believe to be without influence on our results.

BRIEF DISCUSSION OF METHOD

All the protein fraction estimations herein recorded have been made by the refractometric method. The terms 'euglobulin' and 'pseudoglobulin' have the same significance as in our previous papers. The protein fractions which we have separated by fractional precipitation with ammonium sulphate and which we have designated 'albumin', 'pseudoglobulin' and 'euglobulin' are of course not pure substances each containing something of the others. It may, therefore, on a priori

grounds be asked how far quantitative variations of such fractions can form the basis of conclusions of clinical application. It is nevertheless the fact that by adhering to a fixed procedure of precipitation comparative results of considerable clinical value are obtained. A necessary preliminary is the determination of the normals by the method employed. It is very possible that the normals determined by us for human serum and which have been published in previous papers have no rigid accuracy. It is even possible that there are no absolutely fixed normals for these protein fractions in the sense of values independent of the method of precipitation.

We have, for example, been able to show that the changes undergone by these fractions under the influence of treatments specific for the disease in question may be expressed by graphs of fixed type, that in kala-azar the potency of different treatments as suggested by the graphs is in accord with clinical experience, that disturbances on the graphs are associated with variations from the usual prognosis, that indications are provided whether further treatment should be given or withheld, and that the point of cure indicated by the graphs is the point of extinction of the gel reaction with formalin. As will be seen later in this paper an important association with the Wassermann reaction in syphilis has been established. It is claimed, therefore, that the variations determined in the protein fractions thus prepared represent factors of fundamental pathological importance.

We have repeatedly observed that the values obtained by parallel Kjeldahl estimations of the fractions precipitated by anhydrous sodium sulphate agree very closely with those obtained refractometrically after precipitation of the fractions by ammonium sulphate. For the Kjeldahl estimations 30 c c of 14 per cent anhydrous sodium sulphate solution were added to 1 c c of serum (precipitation of euglobulin), and 30 c c of 22.2 per cent anhydrous sodium sulphate solution were added to 1 c c of serum (precipitation of total globulin) following the work of Howe (1921). For the refractometric determinations the euglobulin and total globulin were precipitated by 33 per cent and 50 per cent saturation respectively of ammonium sulphate. Had the above concentrations of anhydrous sodium sulphate been adjusted by Howe so as to produce the same quantitative fractions as those yielded by ammonium sulphate in the above proportions the agreement between the two sets of results, which would be a necessary consequence, would be of no special interest. But the fact is quite otherwise. These concentrations of anhydrous sodium sulphate were arrived at by Howe as those producing critical zones of precipitation. In these circumstances the close agreement of the two sets of figures obtained by using different protein precipitants and entirely different methods of estimation is of great interest. Incidentally it appears to us to dispose of criticisms which have been made as to the accuracy of the refractometric method.

LITERATURE

A study of the literature on the protein changes in syphilis shows that in spite of much experimentation reports based on quantitatively estimated changes of the protein fractions of the blood serum are for the most part vague. It has, of course, been known in a general way for a long time that the globulins in the syphilitic serum are increased, though tests based on this increase are not ordinarily used in clinical medicine. This is in sharp contrast to the use of various tests such as those of Nonne-Appelt, Pandey, etc., to determine the existence of an unestimated increase of globulin in the cerebro-spinal fluid. As the percentage of protein in normal cerebro-spinal fluid is only a mere trace [25 mg per 100 c c according to the recent work of Denis and Ayei (1920)] a definitely positive qualitative reaction with the various protein precipitants is sufficient evidence for clinical purposes of a pathological increase in the cerebro-spinal fluid and quantitative estimations are not usually called for. Tests such as these are not applicable to blood serum which under normal conditions contains approximately 3 per cent of globulin. This is 120 times the total protein content of the normal cerebro-spinal fluid.

The well-known Klausner phenomenon, like the somewhat similar later test of Brahmachari (1917) for the kala-azar serum, is presumably due to an increase of the serum globulin, though Klausner himself (1908) attributed it to the formation of a lipid compound. It is not surprising therefore that it was found not to be peculiar to syphilis and therefore without special value in diagnosis.

Ehas, Neubauer, Poiges and Solomon (1908) thought that the globulins were present in greater amount in syphilitic serum than in normal serum. Spiegler (1908), however, reported a marked diminution of globulin which had no relation to treatment or any clinical factor. These papers are not available in original, but the latter result is quite unlike those obtained by other workers. Noguchi (1909) found an increase of the serum globulin in untreated or slightly treated cases of primary and secondary syphilis.

Rowe (1916) employing the refractometric method found an increase of the serum globulins in syphilis. Tokuda (1921) also found a marked increase of the globulin in syphilis more particularly in active secondary cases. Schiff and Rosen (1920), Bircher and McFarland (1922) and Schmidt (1911) found similar results.

OUR OWN RESULTS

The protein fractions in secondary syphilis

The following table shows the values obtained by us for the various protein fractions in 11 cases of active secondary syphilis —

TABLE

Serum in secondary syphilis (Indians) (females)

Results expressed in grammes per 100 c c of serum

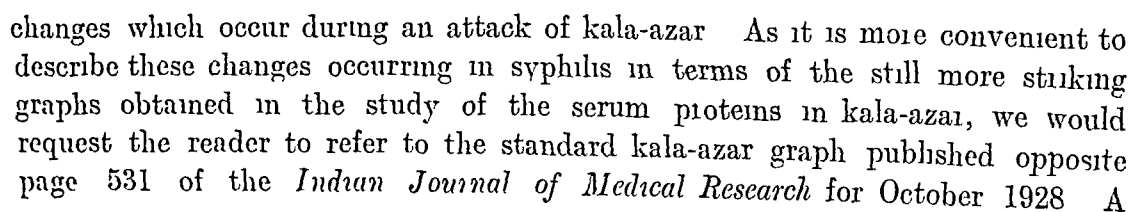
Albumin	Total globulin	Euglobulin	Pseudo globulin	Total protein	Globulin/albumin ratio
3 502	4 122	1 351	2 771	7 624	1 179
3 003	3 414	1 897	1 517	6 417	1 136
3 451	4 256	1 391	2 865	7 707	1 233
3 502	4 122	1 756	2 366	7 624	1 179
3 571	3 902	0 827	2 975	7 553	1 013
3 175	4 373	1 139	3 234	7 548	1 377
3 253	3 665	2 000	1 665	6 918	1 126
2 781	3 061	1 923	1 138	5 842	1 100
3 327	3 813	0 917	2 896	7 140	1 148
3 817	4 217	1 037	3 180	8 034	1 104
3 057	4 780	1 927	2 853	7 837	1 563

It will be seen from the table above that the total serum protein figure in secondary syphilis is usually about the same as that of normal serum, viz, 7.5 grammes per 100 c c. The great change from normal serum is that the globulin has undergone a large increase at the expense of the albumin which has undergone a large decrease. Also the euglobulin has undergone an enormous increase, as much as tenfold in some cases. The great increase of the globulin at the expense of the albumin produces a marked increase of the globulin/albumin ratio to a point far above the normal figure of approximately 0.64. These changes are exactly the same as those seen in kala-azar. We propose to show these changes and the effect of specific treatment thereon by means of graphs as has been done in our previous studies in kala-azar, typhoid fever and malaria. The treatment given is shown at the foot of each graph. It will be observed that the treatment consisted in three cases of N. A. B. and mercurool*, in one case of quinine iodo-bismuthate and in one case of N. A. B. and bismuth (metallic suspension).

* A preparation of mercury nucleinate manufactured by Parke, Davis & Co

The form of the graphs

GRAPH 1

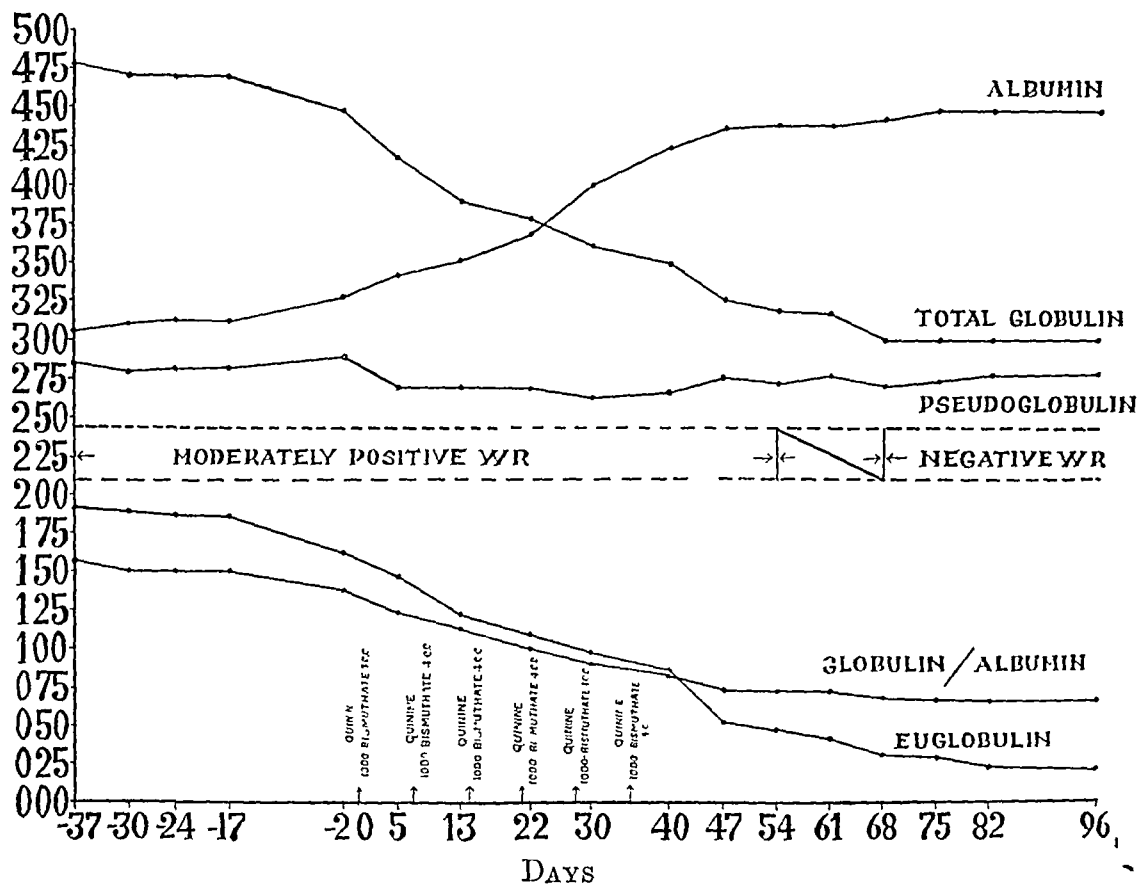


glance shows that the syphilis graph follows the same general course before treatment and exhibits the same type of response to treatment. The chief features are as follows —

(1) In the untreated case of secondary syphilis the total serum protein figure is much the same as that of normal serum, but the albumin and total globulin have changed places, the position again to be reversed under specific treatment with the

GRAPH 2

GRAMMES PER
100 CC SERUM

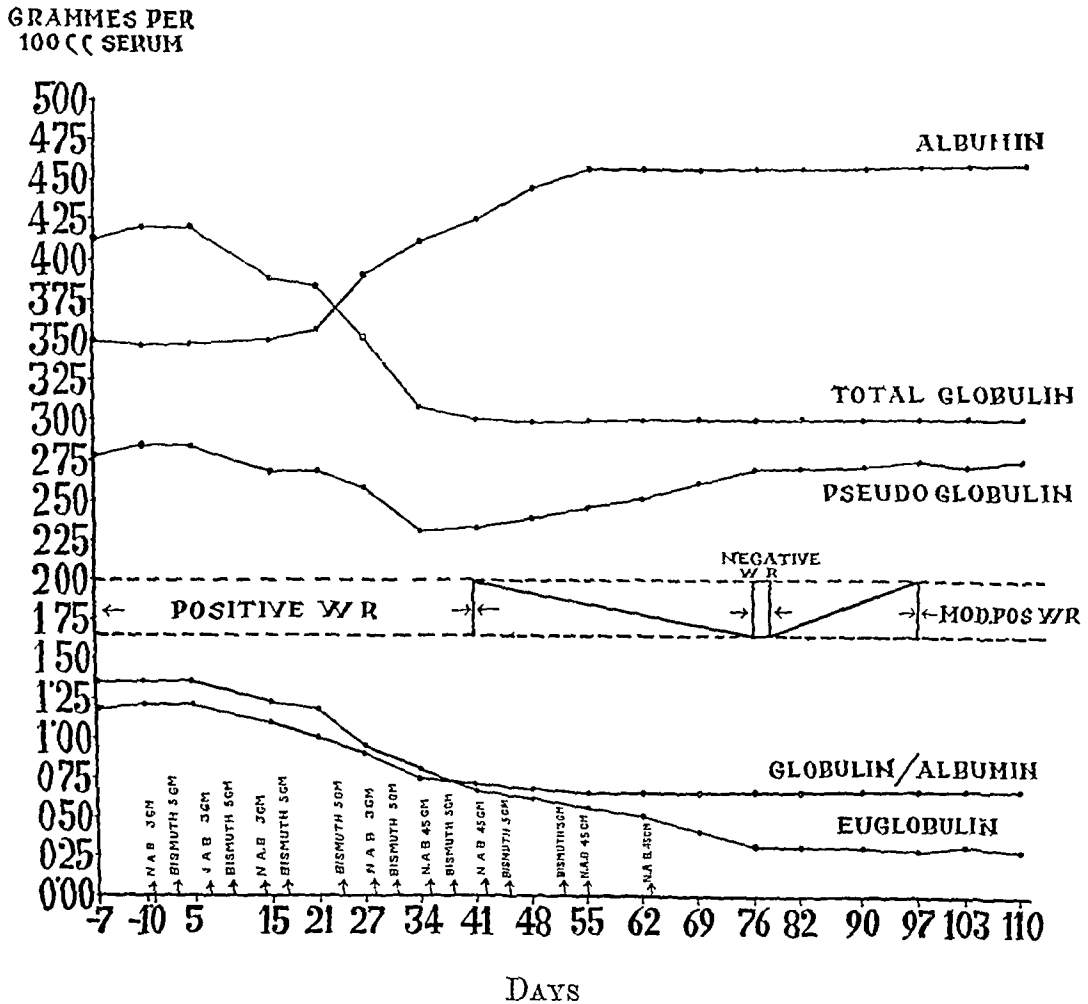


production of the typical X crossing. The same features are shown by the kala-azar graph.

(2) As in kala-azar the graph is definitely divisible into two parts. During the first stage the stabilization of the albumin and total globulin at their normal values takes place and in consequence the globulin/albumin ratio falls to normal. During the second stage the albumin and total globulin values remain steady while

the pseudoglobulin progressively increases at the expense of the euglobulin. There is not in syphilis the sudden drop in the pseudoglobulin and total globulin values which is so characteristic in kala-azar immediately treatment is commenced. The result of this is that the pseudoglobulin and euglobulin curves do not intersect. The upward trend of the pseudoglobulin and corresponding downward trend of the euglobulin can nevertheless be made out as commencing at about the point of

GRAPH 3.



stabilization of the albumin and total globulin values, though not with the same distinctness as in kala-azar

(3) The second stage is only entered in syphilis at about the 48th day of treatment, i.e., approximately twice as long as in a well-established case of kala-azar under treatment by neostibosan

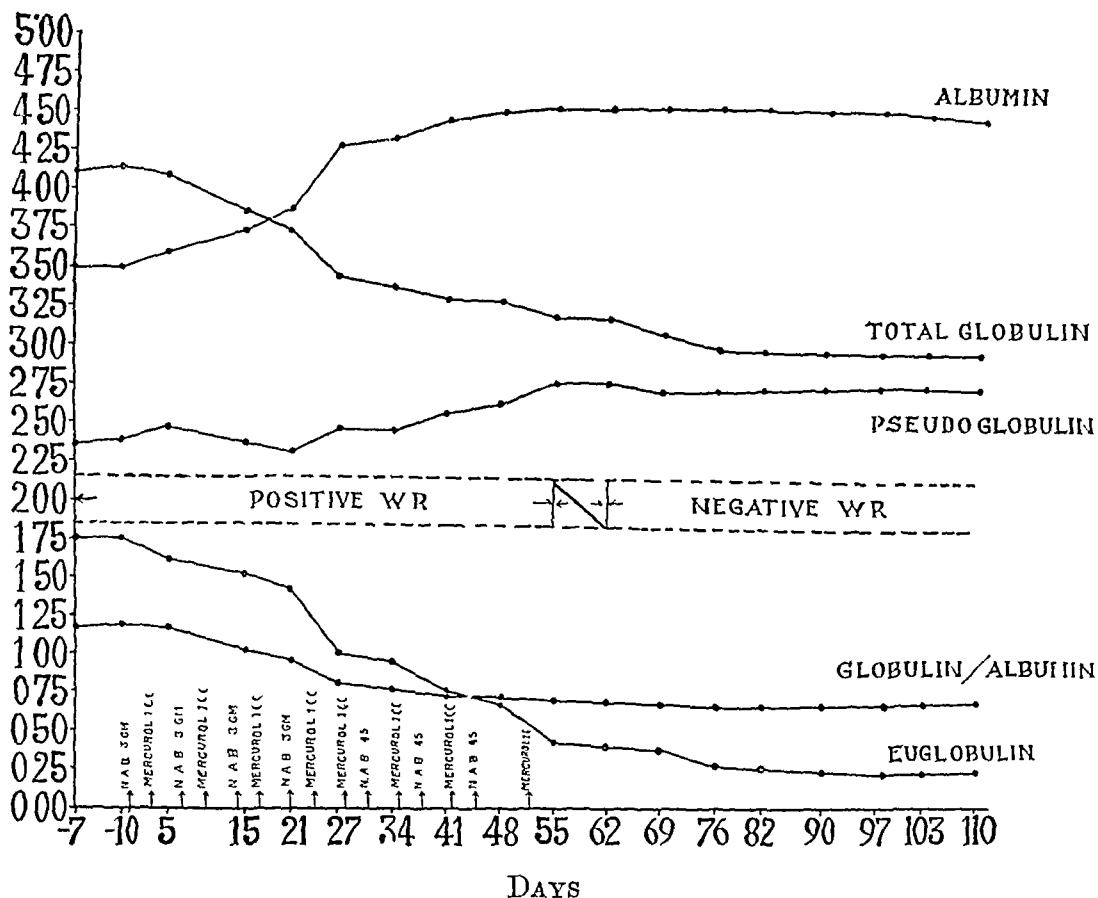
(4) It will be observed that as the first protein phase in secondary syphilis gives place to the second on the 48th day the Wassermann reaction changes from

positive to negative The Wassermann reaction was strongly positive on the 41st day By the 48th day it had weakened to 'moderately positive' On the 53rd day it was still 'moderately positive' and by the 62nd day it had become negative

This graph is quite typical Here the second stage commences on the 68th day of treatment and this was the day on which the Wassermann reaction became for

GRAPH 4

GRAMMES PER
100 CC SERUM



DAYS

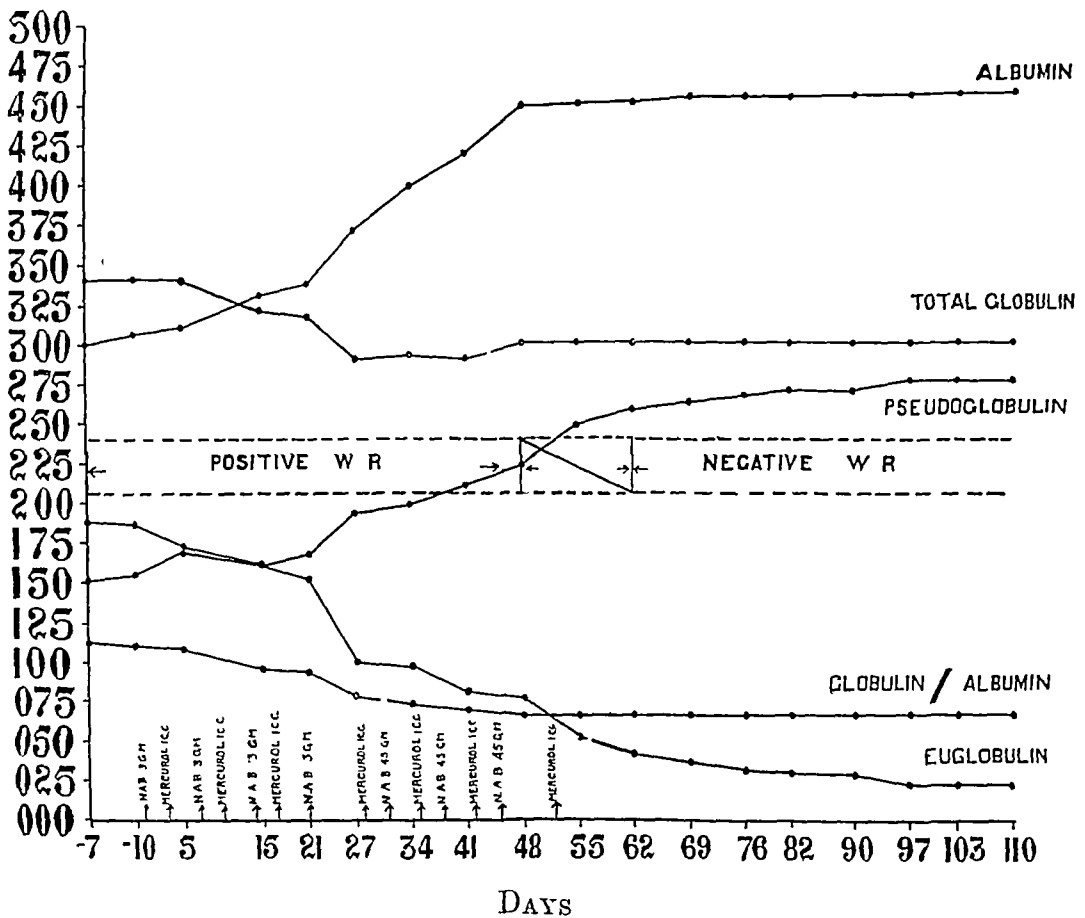
the first time negative The changes in the Wassermann reaction took place between the 54th day and the 68th day It had faded to 'very weakly positive' on the 61st day An interesting feature is that the euglobulin is extremely high and yet the Wassermann reaction was never stronger than 'moderately positive' Also, while the euglobulin fell by the 54th day of treatment to below 0.5 or approximately to one quarter of its value before treatment, the strength of the

Wassermann reaction remained unchanged The slowness of the entry into the second protein phase in this case is of interest in connexion with the treatment given This contained no arsenic

In this case the Wassermann reaction was strongly positive up to the 41st day when it began to weaken, being moderately positive only on the 48th and 55th days, very weakly positive on the 62nd and 69th days, becoming negative for the first

GRAPH 5.

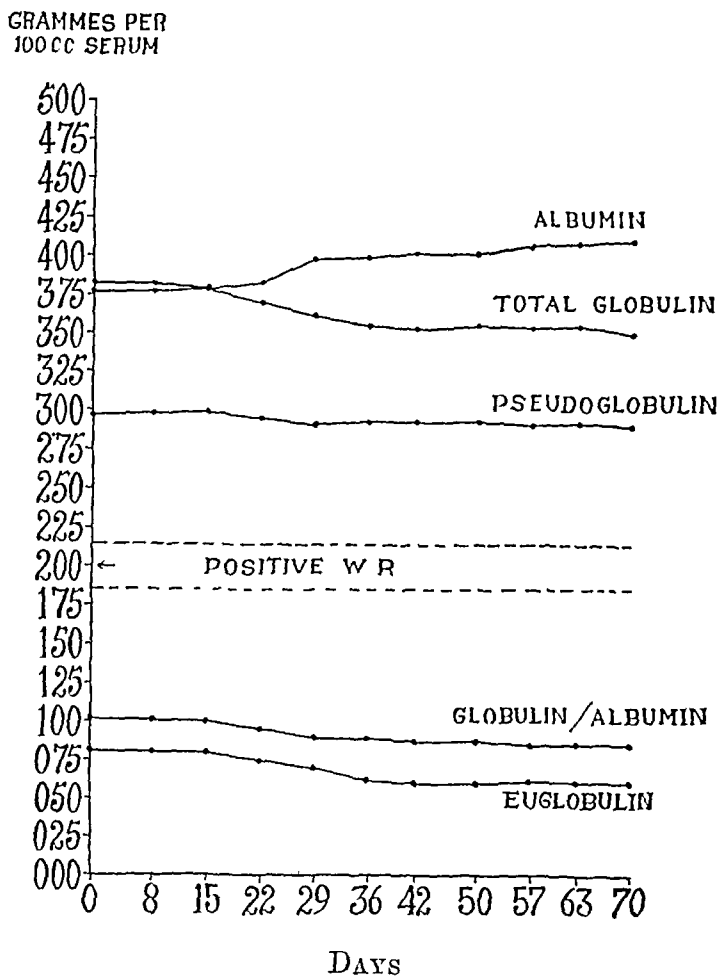
GRAMMES PER
100 cc SERUM



time on the 76th day It will be seen that these changes occurred as the first protein phase was passing into the second at about the 55th day A relapse of the Wassermann reaction to moderately positive had occurred by the 97th and 103rd days Observation on the 103rd day also showed that a slight rise in the euglobulin had occurred which is perhaps not without significance,

In Graph 4 the stabilization of the albumin and total globulin figures is gradual but it had been effected by about the 55th day. The typical divergence of the pseudoglobulin and euglobulin occurs rather earlier than usual. The positive Wassermann reaction, as usual, passes into negative approximately at the point of final stabilization of the albumin and total globulin. The change in the Wassermann reaction was comparatively abrupt in this case, the reaction having been strongly

GRAPH 6



positive on the 55th day becoming negative on the 62nd day. It remained negative subsequently.

In this case the total serum protein is low, the euglobulin being high while the pseudoglobulin is unusually low. As before, the positive Wassermann reaction changes to negative as the first protein phase passes into the second at about the

48th day The Wassermann reaction was strongly positive up to the 48th day It had weakened to moderately positive by the 55th day, becoming negative on the 62nd day It remained negative subsequently Graph 6 was taken from a very hysterical patient with secondary syphilis to whom, during the period in which the graph was taken, no treatment was given Nevertheless a slight movement of the total globulin and albumin occurred towards normality The euglobulin figure is low (0.82) On the other hand the Wassermann reaction remained strongly positive throughout

DISCUSSION

(1) *The effect of treatment*—In our work on kala-azar we suggested that the speed of onset of the second protein phase might serve as an index of the efficiency of the treatment given In well-established cases of kala-azar treated with neostibosan this usually takes about 24 days In syphilis our graphs show that it takes 48 days or longer While we have not as yet had the opportunity of comparing the effect of widely different antisyphilitic treatments on the protein fractions, the cases graphed in this paper were treated in three instances with N A B and mercuriol, in one instance with quinine iodo-bismuthate and in one instance with N A B and bismuth (metallic suspension) These may be taken as fairly representative of modern antisyphilitic treatments On this basis we should conclude that the treatment given in the case of syphilis shown in Graph 1, which was a total of 3.45 grammes of N A B divided into nine injections and eight injections of 1 c.c. each of mercuriol, is considerably less effective than a total of 2.3 grammes of neostibosan divided into eight injections when used in a well-established case of kala-azar, even though in the latter case we are dealing with a disease in which the incubation period is probably very much longer than that of syphilis It will also be observed that the one case of syphilis treated with quinine iodo-bismuthate took considerably longer to enter the second stage than those treated with N A B and mercuriol

If the reader will turn to page 1080 of the *Indian Journal of Medical Research* for April 1929 he will find figured a graph showing protein changes which are entirely typical of syphilis The graph is actually taken from a case of kala-azar under treatment by sodium antimony tartrate The main differences shown by the older remedy in kala-azar as compared with the effect of neostibosan are the slower entrance into the second protein phase and the entire absence of the sudden fall in the pseudoglobulin so characteristic of the modern anti-kala-azar remedies We may in fact describe the typical graph of a case of secondary syphilis under a modern antisyphilitic treatment as being the same as that obtained when a case of kala-azar is treated by sodium antimony tartrate, in that the dramatic fall of the pseudoglobulin produced by neostibosan in kala-azar is absent from both It would seem therefore to be suggested that the new remedies in syphilis, though an immense

improvement on anything available before, are possibly not more efficient than the older treatment of kala-azar, and that the marvellous results produced to-day by neostibosan in kala-azar are not approached by the antisyphilitic courses given in the cases examined. It would seem to follow that there is much room for search for more effective antisyphilitic drugs. There are no doubt essential physico-chemical differences in the serum in syphilis and kala-azar since the one produces a positive Wassermann reaction while the other does not, but failing a better guide it would seem that a search for a drug which would give in syphilis that sudden blow to the pseudoglobulin, so characteristic of the effect of neostibosan in kala-azar would be worth undertaking.

The impression produced by a comparison of the protein graphs in these two diseases that the treatment of kala-azar by pentavalent antimony compounds is considerably superior to the treatment of syphilis by trivalent arsenic compounds is abundantly confirmed by clinical observation. The modern treatment of syphilis is still far from satisfactory in certain respects, more particularly in the length of treatment required. On the other hand we have in neostibosan a drug which by a series of injections compressed into one week can effect a cure in over 90 per cent of cases of kala-azar. While we are still very much in the dark as to the mode of action of these specific drugs, it would seem probable from the study of the graphs that the mechanism of cure both in syphilis and kala-azar is bound up with the ultimate conversion of more and more of the euglobulin into the water-soluble form (pseudoglobulin). This process apparently must be preceded by a normal protein ratio which is brought about by the stabilization of the albumin and total globulin fractions at their normal values, an important feature of which appears to be the conversion of pseudoglobulin into albumin.

As regards the question as to whether diseases of this type should invariably be treated at the earliest possible moment, the joint clinical and serological study of kala-azar by Napier and ourselves rather suggested that in the absence of acute symptoms there need be no hurry to commence treatment in that disease and that waiting might even improve the prognosis. It seemed to us possible that the globulin rise in kala-azar was in some sense a defensive process which might assist the subsequent response to medication. In syphilis, on the other hand, the opinion of the very large majority of clinicians is that treatment should be commenced at the very earliest moment the diagnosis is definitely made, though not of course before. General experience seems to show that 'sero-negative' cases of syphilis, i.e., those in which treatment is commenced before the Wassermann reaction has had time to become positive, give the best results, and it is accepted clinical teaching that if the Wassermann reaction can be kept negative for the first year the outlook is usually good. It is also well known that old cases with a positive Wassermann reaction even without visible lesions usually have an unfavourable ending caused by visceral lesions, e.g., aortitis. Closely connected with this matter is the question

whether the action of specific drugs is direct on the parasites or indirect by means of body resistances Hazen is alone among leading syphilologists in believing that the Wassermann-positive cases show better end-results than the 'sero-negative' cases This opinion may yet be brought into line with Taniguchi's view (1924) that the positive Wassermann reaction is an immunity response against a lipoid antigen

It will be seen from the graphs that the positive Wassermann reaction passes into the negative reaction as the first protein phase passes into the second This is an important matter which will be referred to again later

(2) *The existence of protein fractions in the living blood* —We have spoken above of the process of cure in syphilis as being connected with a progressively increasing proportion of the total globulin assuming a water-soluble form (pseudoglobulin). This implies the existence of these protein fractions in the living blood It is necessary therefore to state that according to the views of certain workers, e.g., Svedberg and Sjogren (1928) the fractions euglobulin and pseudoglobulin have no existence in the living blood, being only laboratory products This is an obscure question, but if this view be accepted it is clear that we must regard the change we have detected during the stage of cure both in syphilis and kala-azar as a change in an assumed homogeneous colloidal solution of globulin whereby it becomes less and less precipitable by ammonium sulphate or other similar electrolyte in concentrated solution The corollary of this view would appear to be that in a disease like syphilis the degree of dispersion in the colloidal solution is reduced by a coalescence of the smaller particles (albumin) to form the larger complexes (globulin) On this view one would expect that the amount of protein thrown out of solution by protein precipitants in fixed proportion (e.g., ammonium sulphate to 33 per cent saturation) would be greater from the syphilitic serum than from the normal, the early stages of flocculation having really occurred *in vivo* On the other hand, the immunological evidence of the existence of these fractions is strong Thus Hektoen and Welker (1924) successfully prepared precipitin antisera against euglobulin and pseudoglobulin from many different sources Each of these antisera reacted specifically with its homologous protein fraction, and, what is essential to our discussion, reacted also with the whole serum from which the protein fractions were derived This observation would be accepted by all immunologists as sufficient proof that the antigen in question exists in the solution under test The presence of the protein fractions is therefore demonstrable in whole serum to which no protein precipitants have been added The fractions thus retain their antigenic independence in the serum Dale and Hartley (1916) arrived at somewhat similar conclusions and showed that 'each of the three proteins separable from horse serum by their physical and chemical properties—euglobulin, pseudoglobulin and albumin—can act as an anaphylactic antigen'.

Landsteiner and Doerr take up a position intermediate between these two views in stating their opinion that small chemical differences exist between the single protein fractions, though they qualify their statement by adding that a transition 'fließende Uebergang' takes place between the single fractions

In a previous paper we put forward the view that the pseudoglobulin is the pivotal centre of a system in which (a) during the development of the disease pseudoglobulin is converted into euglobulin, (b) that during the first protein phase initiated by treatment pseudoglobulin is converted into albumin, and (c) that the second protein phase induced by treatment is a process of conversion of euglobulin into pseudoglobulin. There would apparently be no difficulty in accommodating this conception in the scheme of things visualized by Landsteiner.

(3) *The connexion between the Wassermann reaction and the protein phases in syphilis*—This paper is not concerned with the ultimate cause of the Wassermann reaction. We therefore do not propose to refer to the vast amount of experimentation which has been undertaken to discover its nature, the more so as these researches have been somewhat inconclusive. We propose to limit ourselves here almost entirely to a discussion of the time relations of the Wassermann reaction *vis-à-vis* the protein changes, and certain consequences which appear to follow. The graphs show that in secondary syphilis the positive Wassermann reaction changes over to negative as the first protein phase passes into the second. It would appear, therefore, that there is some association of a quantitative nature between the Wassermann result and the protein fractions. Several of the graphs show that it is not the absolute value of the euglobulin which determines the outcome of a Wassermann reaction. Since the pseudoglobulin and euglobulin curves pursue opposite paths during the second protein phase, we examined the quantitative relation between these two fractions at the commencement of this phase without finding any correlation. The second protein phase implies by definition that the stabilization of the albumin and total globulin at their final normal values has already occurred, though at its commencement the total globulin contains much more euglobulin and correspondingly less pseudoglobulin than does the globulin of the normal serum. A study of the graphs shows that the change in the Wassermann reaction takes place as the total globulin and albumin stabilize themselves at their final values. At the commencement of the second phase we thus find a normal/globulin albumin ratio and a negative Wassermann reaction. As the change in the Wassermann reaction from positive to negative precedes the protein changes characteristic of the second phase—the process by which a progressively increasing proportion of the globulin becomes water-soluble—it is therefore not dependent thereon. The graphs therefore suggest that it may be the rise of the albumin to its normal excess over the globulin which extinguishes the Wassermann reaction, i.e., that the albumin acts as a check inhibiting the mutual precipitation which takes place between the antigen and the syphilitic serum in the first stage of the

positive Wassermann reaction As stated, the cause of the positive Wassermann reaction is not definitely known The view of Friedemann (1910) that the factor responsible is a globulin-soap compound towards which the serum albumin is antagonistic has many adherents Schmidt (1911) believes the positive reaction due to changed reaction powers of the globulins in syphilis whereby they display enhanced affinity for the colloids of the antigen, this action being held in check by the albumin which in syphilis is reduced

It will be seen that the conclusions directly deducible from the graphs are in accord with the above views of Friedemann and Schmidt If our view of the dependence of the Wassermann result in syphilis upon the protein phase is correct, it follows that we cannot have a positive Wassermann reaction in a syphilitic serum with a normal protein ratio

Since in tertiary syphilis it is the rule to find a positive Wassermann reaction and these positive reactions may sometimes be obtained in cases which have previously been rendered Wassermann-negative by specific treatment, it would be of great interest to examine whether the protein ratio has relapsed in association with the relapse of the Wassermann result We have not as yet had any opportunity of determining this point As distinct from Wassermann relapses in properly treated cases there is naturally no opportunity of following cases which have been neglected from the first right up to the point where tertiary lesions are seen In view of the well-known obstinacy of the positive Wassermann reaction in neglected cases, the possibility exists that the treatment was not sufficiently effective to induce the onset of the second protein phase If my view of the invariable association of the first protein phase with the positive Wassermann reaction and the second protein phase with the negative reaction is correct, old Wassermann-fast cases of syphilis should invariably show an abnormal protein ratio If this proves to be so, the term Wassermann-fast will acquire a greatly increased significance

To test this view further we examined the protein fractions in six old tertiary cases to ascertain (a) if they were in the first protein phase, even though, were this the case, we could not tell from the impossibility of continuous observations whether they had at one time been in the second phase and had relapsed, or whether they had owing to the absence or insufficiency of treatment always been in the first phase, (b) if the protein fractions reacted to treatment in the same manner as those of the secondary cases, and (c) if the same association with the Wassermann reaction held good .

For the sake of brevity we will omit full clinical details of these old cases, but they were cases of (1) obviously syphilitic perforation of palate—no history of syphilis, (2) extensive gummatous periostitis of tibia—syphilis 18 years ago, (3) generalised adenitis—syphilis 6 years ago, (4) extensive and active tertiary lesions of the skin—syphilis 17 years ago, (5) gumma of sternum—syphilis 30 years ago, (6) gumma of nose—syphilis 15 years ago All, except the last who had received

efficient adequate treatment shortly before we commenced observations, had a strongly positive Wassermann reaction

We show here two graphs (Graphs 7 and 8) to illustrate the protein fraction condition in the old tertiary cases. It will be seen that before treatment both are in the first protein phase since the globulin/albumin ratio is above normal, and that under treatment exactly the same sequence of changes occurs as in the secondary cases, the positive Wassermann reaction passing into negative as the first protein phase gives place to the second. In Graph 7, which was taken from a case of gumma of sternum (syphilis 30 years ago), the albumin is, unlike the condition in the untreated secondary case, higher than the globulin. Under treatment the albumin rises still further while the globulin falls, the normal globulin/albumin ratio being reached on the 28th day of treatment.

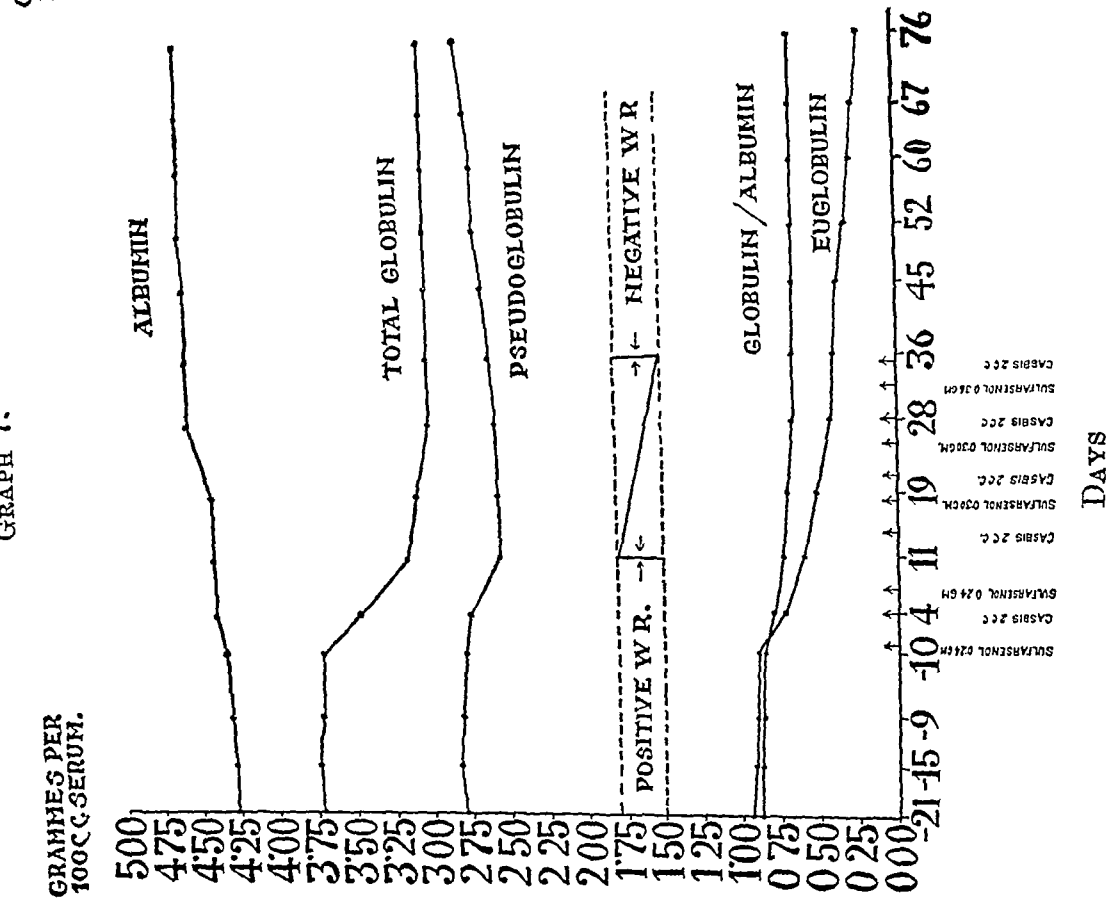
In Graph 8 which was taken from a case of extensive gummatous periostitis of tibia (syphilis 18 years ago) the globulin is still slightly higher than the albumin and the X crossing is just visible. Under treatment the normal globulin/albumin ratio and the negative Wassermann reaction are reached on the 42nd day. As shown in the graphs the treatment given to these two old cases consisted of intra-gluteal injections of sulpharsenol and casbis*. This treatment proved remarkably effective.

Graphs 7 and 8 show that these tertiary cases were before treatment in the latter part of the first protein phase.

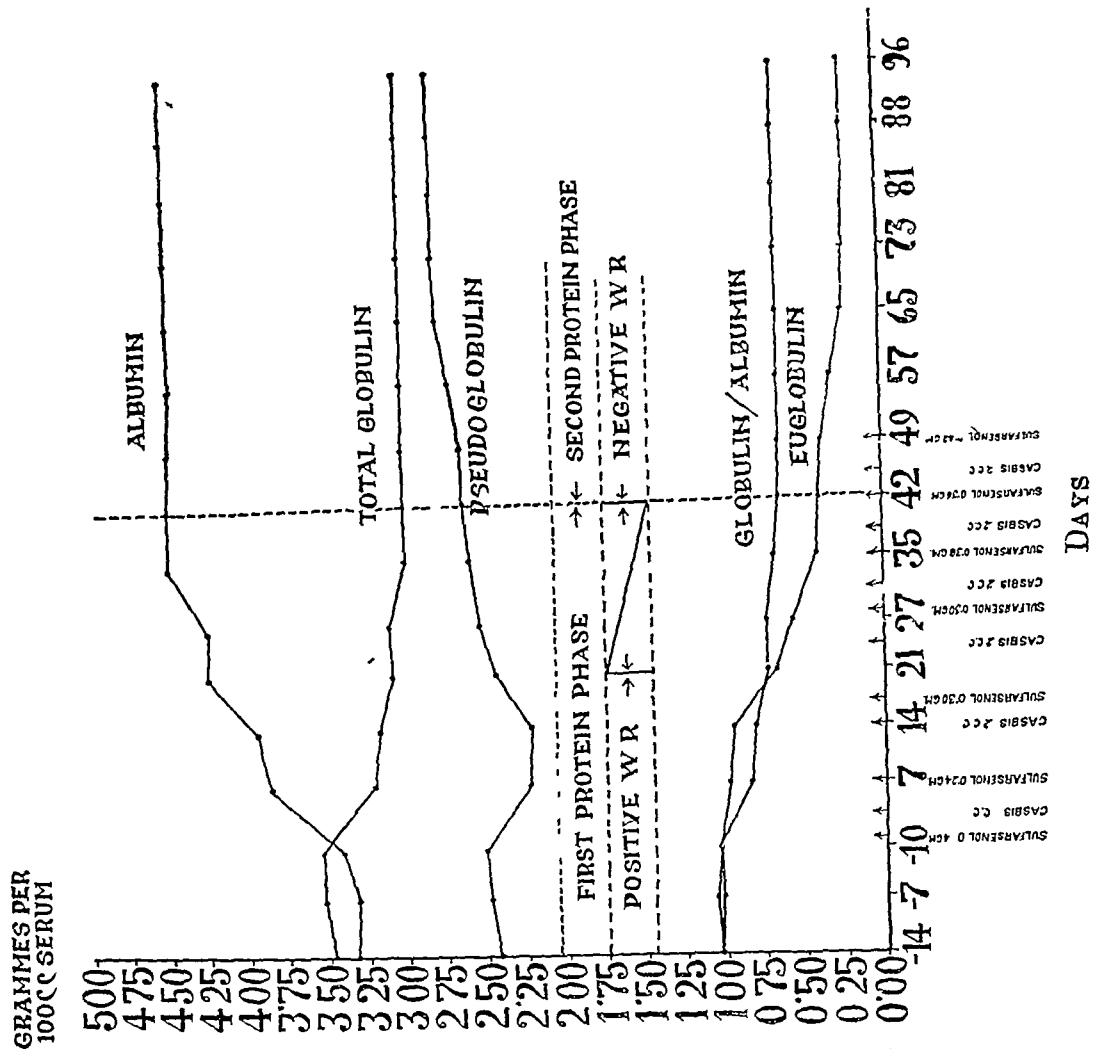
The limitation of the positive Wassermann reaction to the period of the first protein phase thus holds good for the old cases as well as for the early ones. The whole of the second protein phase is therefore a post-Wassermann phase of protein abnormality. It follows that by means of the protein fraction graph we can see further into the syphilis patient under treatment than is possible by means of the Wassermann reaction. Graph 8 shows this particularly well. This immediately raises the question as to whether there is a pre-Wassermann stage of protein abnormality in syphilis. Up to the present we have not been able to obtain much information on this point. This is a matter of very great practical importance in connexion with early diagnosis. The Wassermann reaction frequently does not become positive in less than six weeks after infection, and if the practitioner has to deal with a sore of suspicious origin in which the treponema has not been found he is placed in a position of some difficulty, since he wishes to start the treatment as early as possible but not on a doubtful diagnosis. If a pre-Wassermann phase of protein abnormality exists it could be utilized as a means of differentiating an infecting sore from others, and would in this way be of great service. This question will be examined later.

* An emulsion of activated bismuth hydrate containing 0.1 g bismuth in one c.c. manufactured by I. G. Farbenindustrie, Leverkusen.

GRAPH 7.



GRAPH 8.



If further experience should confirm our present views it seems that we may perhaps in the later examinations of the blood of a syphilitic be able to substitute with advantage the refractometric determination of the protein fractions for the more complex procedure of the Wassermann reaction. No protein fraction estimation can decide whether a case is one of syphilis or not, since the high globulin first phase of syphilis is met with in kala-azar and probably in other conditions. Once however the case is definitely diagnosed to be one of syphilis by a combination of clinical signs and a positive Wassermann reaction, its progress under treatment during the later stages may probably be just as well gauged by the direction of the pseudoglobulin and euglobulin curves as by the Wassermann reaction. We have suggested above that, as an index of progress under treatment and final cure, the protein fraction determination may prove to be the more delicate test of the two, since the graphs show that there is a final stage in which the assumed protective action of the albumin is in full operation, as shown by a normal globulin/albumin ratio and a negative Wassermann reaction, yet an abnormal quantitative relation still exists between the pseudoglobulin and euglobulin. The above considerations do not imply that the sole cause of the positive Wassermann reaction in syphilis is to be found in protein fraction changes, since those found in kala-azar are practically the same. In view of this we considered it important to examine the Wassermann reaction in kala-azar, and we published the results of examination of the large series of 474 cases (Lloyd, Napier and Mitia, 1930). These showed no evidence that kala azar is a cause of a positive Wassermann reaction. It seems clear therefore that quantitative changes in the protein fractions in a non-syphilitic disease will not alone generate a positive Wassermann reaction. It does, however, appear to be the case that, given the special circumstances met with in syphilis, the quantitative relations between the protein fractions determine the Wassermann result.

The idea also suggests itself as to whether we may not from consideration of the protein fraction changes throw at least some light on the positive Wassermann reactions which are reported by certain workers to occur in malaria by the use of certain Wassermann techniques. We cannot tell whether the special circumstances found in the syphilitic serum may not obtain to a limited extent in other diseases. We have, however, elsewhere shown that, while the globulin in malaria undergoes but little quantitative change, the albumin is markedly reduced (Lloyd and Paul, 1929). If the albumin acts as a mechanism antagonistic to the positive Wassermann reaction, we might expect that given the preliminary essential serum change, there would be a positive Wassermann reaction in malaria. We do not by the use of our own Wassermann method find any positive Wassermann reactions in malaria (Lloyd and Mitia, 1926) other than those definitely attributable to concomitant syphilis, and we regard reports of such positive reactions in malaria as an indication

of the use of over-sensitive techniques which are contraindicated in the tropics where the exclusion of positive fixations due to malaria is an essential control. No reports are available as to the Wassermann reactions of typhoid fever and kala-azar sera when tested by techniques sufficiently supersensitized to yield positive reactions in malaria.

CONCLUSIONS

(1) A series of protein fraction graphs taken from cases of secondary syphilis is given. These show marked general resemblance to those obtained in kala-azar, the characteristic features being less strongly marked in the former disease.

(2) From certain differences in the graphs in the two diseases some theoretical aspects of treatment in syphilis are discussed from the standpoint of the more favourable results obtained in kala-azar.

(3) It is shown that in cases of secondary syphilis treated on modern lines the positive Wassermann reaction changes to negative as the first protein phase passes into the second.

(4) It is concluded that, given a serum from a case of syphilis, the question as to whether it will react positively or negatively to the Wassermann test is decided by the quantitative relation between the total globulin and the albumin, i.e., whether the globulin/albumin ratio is above normal or not. This conclusion rests upon the association of the positive Wassermann reaction with the first protein phase, the negative Wassermann reaction being associated with the second protein phase. We believe that the albumin acts as a preventive of the Wassermann reaction which is therefore only positive in the first protein phase during which the albumin is greatly reduced. When the albumin rises in the second phase to its normal excess over the globulin its protective action reasserts itself and the Wassermann reaction becomes negative again.

(5) No protein fraction examination can diagnose a case to be one of syphilis, since practically the same changes are met with in kala-azar.

(6) We suggest, however, that given a definitely diagnosed case of early syphilis its progress under treatment may probably be just as well gauged by the direction of the pseudoglobulin and euglobulin curves during the second protein phase as by the more complex procedure of the Wassermann reaction. It is possible that the protein graph test may prove to be the more delicate of the two, since it detects the later change of the progressively increasing solubility of the globulin, whereas the Wassermann reaction has become negative before this process commences. There is thus a definite post-Wassermann phase of protein abnormality.

(7) No evidence has yet been obtained of the existence of a pre-Wassermann phase of protein abnormality in syphilis. This is a matter of considerable importance in connexion with early diagnosis.

(8) Observations carried out on cases with late lesions show that they are in the first protein phase as the globulin/albumin ratio is above normal and the Wassermann reaction positive. The graphs of the old cases also show that the protein fraction response to treatment is exactly the same as that of the secondary cases and that exactly the same association exists between the Wassermann result and the protein phase. The existence of the post-Wassermann phase of protein abnormality in the late cases also is thus demonstrated. The conclusion that the protein fraction test can follow the cure process further than is possible by means of the Wassermann reaction appears to be just as valid for the old cases as for the early cases.

(9) The suggestion is tentatively put forward that the reduction of the albumin which occurs in malaria may possibly play some part in the causation of the positive Wassermann reactions in that disease which have been reported by workers with certain techniques.

ACKNOWLEDGMENTS

I desire especially to thank my assistant, Mr S N Paul, M Sc, for carrying out the refractometric observations.

For the use of clinical material freely accorded to us grateful acknowledgment is made to Lieut-Colonel A Denham White, I M S, Superintendent of the Voluntary Lock Hospital, Alipore, Calcutta, and his staff. I am also indebted to the outpatient staff of the Medical College Hospital, Calcutta, for carrying out the treatment of the six old cases under special observation.

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A NEW AND CHEAP SOURCE OF UREASE

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[Received for publication, December 4 1931]

THE source of urease generally employed in all laboratories is the soya bean or the jack bean. As pointed out by Sumner (1928) these beans are variable as regards their activity, and during the course of our investigations we have tested at least two samples that showed very little or no activity. We have been investigating the possible local sources of urease with a view to find a cheap and readily available source of the enzyme which will yield a sufficiently potent preparation for the routine clinical work. Of the seeds so far examined, two have been found which contain urease, namely, the common horse gram (*Dolichos biflorus*) and dhal (*Cajanus indicus*). Of these the horse gram appears to be very efficient, comparing favourably with many samples of soya bean, and promises to be a cheap and reliable substitute for the latter. This paper deals with a study of its claim to recognition as a source of urease by biochemists.

EXPERIMENTAL

Activity of Dolichos biflorus compared with that of soya bean at different temperatures

A comparative study was made of the activity of random specimens of *Dolichos biflorus* obtained from different shops in the local market with a sample of soya bean got from Messrs Griffin and Tatlock about a year ago, which is the best we have, at temperatures ranging from 40° C to 90° C. This was suggested by the consistent observation of more intense and more rapid production of red colour with phenol red when gram powder is added to a fresh solution of urea as compared with the colour produced by adding an equal quantity of soya bean powder to an equal

(1077)

volume of urea solution Further, every specimen of *Dolichos biflorus* was found to be more active than soya bean when tested by the following method which was designed by us to rapidly compare the urease activity of different specimens The method, similar to Wohlgemuth's technique of estimating diastase (Cole, 1928), is as follows The unit of urease adopted by us is the activity contained in the minimum amount of material which is capable of completely decomposing 1 mg of urea in 15 minutes at 60° C

Rapid method of testing urease activity—Into a series of test-tubes, numbered and arranged in a rack, are added varying amounts of urease solution and water as indicated in the table below The urease solution is prepared from the material to be tested in exactly the same way as stated in the subsequent portion of this paper, i e, 1 g of the powdered material extracted with 16 per cent alcohol for 5 minutes, the whole being made up to 50 c c and filtered

Tube	Urease solution, c c	Water, c c	Tube	Urease solution, c c	Water, c c	Tube	Dilute urease 1 in 10	Water, c c
1	1 0	0 0	6	0 5	0 5	11	0 9*	0 1
2	0 9	0 1	7	0 4	0 6	12	0 8	0 2
3	0 8	0 2	8	0 3	0 7	13	0 7	0 3
4	0 7	0 3	9	0 2	0 8	14	0 6	0 4
5	0 6	0 4	10	0 1	0 9	15	0 5	0 5

Thus the volume in each case is 1 c c with urease in decreasing concentration To each tube is added 1 c c of buffered urea solution (1 c c = 1 mg) commencing from the end having the lowest concentration of urease All the tubes are then incubated at 60° C for 15 minutes, at the end of which time they are simultaneously plunged into boiling water and kept there for 5 minutes The tubes are now cooled by immersion in cold water, and two drops of phenol red indicator is added to each tube The contents of all the tubes turn pink Next very dilute acetic acid (0 1 per cent) is added drop by drop till the colour just turns yellow One c c of urease solution is again added to all the tubes, and the latter incubated at 60° C for 5 to 10 minutes The tubes which originally contained undecomposed urea now show a pink colour The last of the tubes which are yellow contains just sufficient urease to decompose 1 mg of urea under the given conditions Thus, if it is the sixth tube in the series, which contains 0 5 c c urease solution representing $\frac{1,000}{50 \times 2}$ or 10 mg of the original powder, the urease activity of the latter will be 100 units per gramme

* The same urease solution diluted ten times with water

of material Tested by this method Dolichos powder had an activity of 126 units as against 83.3 units for soya bean This led us to investigate whether this superior activity is maintained at all temperatures under well-controlled conditions

As will be evident from the following data the urease activity of horse gram is decidedly greater than that of the sample of soya bean at all temperatures between 40° C and 90° C In each case 1 g of the fine powder was extracted with 16 per cent alcohol for 5 minutes by grinding in a glass mortar, at the end of which period the whole suspension was transferred into a graduated cylinder, and the volume made up to 50 c c with repeated washings of the mortar with 16 per cent alcohol The contents were well mixed, and then filtered through ordinary filter paper Two c c of the opalescent filtrate was measured into a test-tube 200 mm × 20 mm forming part of Van Slyke's apparatus for estimating urea by the aeration method (Cole, 1928) The test-tube was kept in a bath at the required temperature, and 5 c c of 3 per cent buffered urea solution, also brought to the same temperature, was added and the tube fitted to the aeration apparatus, a very slow current of air being drawn through The urea solution used had the same composition as that used by Sumner (1928) having a pH slightly on the acid side of the neutral point, as we found that the enzyme acts better at this reaction than at pH 7.0 The action

TABLE I

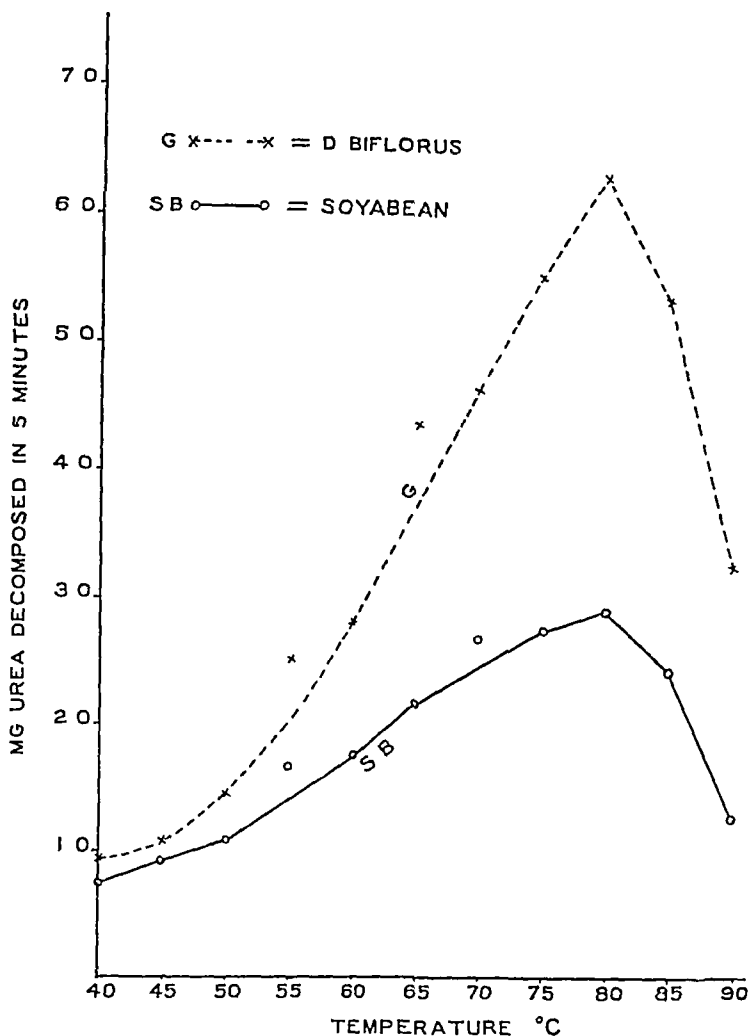
Showing the amount of urea decomposed in 5 minutes at different temperatures by 2 c c of extract of Dolichos biflorus and soya bean, and the comparative activity of each

Temperature, °C	UREA DECOMPOSED IN 5 MINUTES BY 2 C C EXTRACT, MG		K × 10 ³	
	<i>D. biflorus</i>	Soya bean	<i>D. biflorus</i>	Soya bean
40	0.954	0.749	1.334	0.92
45	1.056	0.939	1.426	1.196
50	1.436	1.064	2.392	1.426
55	2.548	1.649	3.358	2.116
60	2.823	1.772	3.772	2.392
65	4.358	2.150	5.934	2.806
70	4.613	2.676	6.210	3.634
75	5.490	2.735	7.452	3.634
80	6.274	2.881	8.602	3.910
85	5.309	2.413	7.176	3.220
90	3.232	1.272	3.358	1.702

was allowed to proceed for 5 minutes, at the end of which it was suddenly arrested by adding 3 c c normal sulphuric acid by way of the inlet tube, at the same time introducing a few drops of caprylic alcohol, after which a strong current of air was drawn through for 1 minute, which served to mix the acid well with the contents of

Curves showing the relative activity of Dolichos biflorus and soya bean at different temperatures

GRAPH



the tube The amount of urea decomposed in 5 minutes was determined by the method of Van Slyke (Cole, 1928), using 0.01 N acid and 0.01 N alkali The same procedure was adopted for each experiment separately Similar experiments under identical conditions were done with soya bean The data are given in Table I The curves are drawn from these results

Dolichos biflorus as a substitute for soya bean

The efficiency of *D biflorus* for the quantitative determination of urea in solutions of known concentration as well as in blood and other biological fluids was studied in comparison with that of soya bean. In all cases the determination of the amount of urea decomposed was done by Van Slyke's method (Cole, 1928) incubating at 60° C for 15 minutes and using 0.01 N acid and 0.01 N alkali. In every instance it was found to be at least as efficient as soya bean, as will be seen from Tables II and III —

TABLE II

Showing the decomposition of urea solutions of known strength by D biflorus and soya bean

	URFA PHOSPHATE SOLUTION AND STRENGTH,		Urea determined, mg	Urea calculated, mg	Error, mg
	c c	per cent			
Soya bean	3	0.2	5.375*	6.0	-0.625
<i>D biflorus</i>	3	0.2	5.885	6.0	-0.115
Soya bean	3	0.1	3.059	3.0	+0.059
<i>D biflorus</i>	3	0.1	3.293	3.0	+0.293
Soya bean	1	3.0	30.379	30.0	+0.379
<i>D biflorus</i>	1	3.0	29.661	30.0	-0.339
Soya bean	5	0.1	5.049	5.0	+0.049
<i>D biflorus</i>	5	0.1	5.180	5.0	+0.180
Soya bean	0.5	3.0	15.964	15.0	+0.964
<i>D biflorus</i>	0.5	3.0	15.438	15.0	+0.438

* The figures are the average of duplicate estimations.

TABLE III

Showing comparative results of urea estimation in biological fluids

	MG PER 100 C C FLUID		REMARKS
	Soya bean	<i>D biflorus</i>	
<i>Blood</i>			
P A	30 80	30 80	Normal blood
V E	29 83	29 83	„ „
B R S	33 73	32 73	„ „
G V	26 00	26 00	Beri beri
A S		273 00	Uremia N P N 146 mg Andrew Hewit's reaction positive
<i>Urine</i>			
D N R	808 53	901 75	
N A	608 65	614 40	
<i>Cerebro spinal fluid</i>			
A S		349 00	Uræmia Same case as above Lumbar puncture done the day after taking the blood N P N 144 6 mg

During the course of these quantitative experiments it was incidentally observed that addition of an excess of the gram powder tends to give a slightly higher figure than the theoretical, suggesting the possibility of the presence of free amide groups in the constitution of the substance. Hence the content of amide nitrogen was determined in samples of the powder by adding sufficient alkali to 1 g of the powder and aspirating ammonia-free air through it into 10 c c water containing 0.5 c c Folin's digestion mixture for micro-kjeldahl. At the end of aeration the acid solution was transferred into a 50 c c volumetric flask, the volume made up to 30 c c with two washings of 10 c c each, 15 c c of Folin's modified Nessler's solution (Beaumont and Dodds, 1929) added, and the volume finally made up to 50 c c. A standard was prepared in the same way as for the determination of non-protein nitrogen by Folin's method (Beaumont and Dodds, 1929), and the unknown solution was compared in a colorimeter with the standard at 20 mm $\frac{300}{\text{Reading of unknown}}$ gives number of mg of amide nitrogen per 100 g of powder. Determined

in this way *D. biflorus* contains on an average 29.7 mg of amide nitrogen per 100 g of powder. However, with the quantity of powder ordinarily used for a urea determination the error from this cause cannot be very significant. Further, it has been observed that this source of error can be completely eliminated by using the powdered material obtained by precipitation with alcohol of a thymol-water or dilute alcohol extract of *Dolichos* powder in place of the crude material. This has the additional advantage of being more active weight for weight than the crude powder. Work on the extraction and purification of the enzyme from this source is now in progress.

In conclusion we hope that sufficient evidence has been presented in this paper to warrant an extensive use of *Dolichos biflorus*, a readily available, unfailing, and economically a much cheaper material, as a source of urease in place of soya bean.

SUMMARY

- 1 Two new sources of urease have been found.
- 2 *Dolichos biflorus*, the ordinary horse gram, is a cheaper than and an equally efficient source of urease as soya bean.

We wish to express our thanks to Lieut-Colonel F. J. Anderson, I.M.S., the Principal of the College, and to Dr. B. B. Dikshit, Professor of Pharmacology, for the encouragement they gave us.

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COMPARATIVE STUDY OF LARVAL CHARACTERS OF
A LUDLOWII (THEOBALD) AND
A SUBPICTUS (GRASSI).

BY

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[Received for publication, December 14 1931]

At a conference of experts to consider the gravity of the menace of malaria to Calcutta and its Port from the invasion of *Anopheles ludlowii*, it was decided to institute an enquiry into its distribution and to find out the causes of such distribution if possible. The Government of Bengal taking up the suggestion of the experts sanctioned a staff for the survey. The author of this article was put in its charge.

The author was very much handicapped in the beginning of the survey work as the larval characters of *A ludlowii* hitherto described by various authors were quite inadequate for the purpose of identification of the species in their larval stage. Full description of *A ludlowii* larva given by Swellengrebel in his famous book (1919) and in the *Bull Ent Res*, 11, 72-92 (1920), does not reveal any differentiating points between *A ludlowii* and *A subpictus*. In Strickland's book also the differentiating points between the two species are not mentioned. And, lastly, the points of identification between *A ludlowii* and *A subpictus* as recently given by Puri (June, 1931) in his admirable treatise dealing with larval character of Indian species of Anopheline mosquitoes could not be utilized to any effective purpose as they are in his own opinion not reliable.

Hence to find out the prevalence of *A ludlowii*, collection of a large number of adult mosquitoes was made and then villages showing this species were critically examined to ascertain the breeding places. There being no distinguishing characters described by previous workers, thousands of bottles had to be kept for the final hatching out of the larvæ with characters of salt-breeding *A subpictus*. Subsequently a thorough and careful search was made to find out some point or points of difference between these two species of larvæ, which present almost identical characters.

For this purpose a large number of larvæ were collected from infected villages and bred out at the laboratory. The majority of them, when hatched out, consisted mainly of *A. ludlowi* with a smaller number of *A. subpictus* and *A. vagus*. The hatched out adults of *A. ludlowi* were reared in the laboratory with regular blood-meals and the larvæ hatched from eggs of these examined in large numbers along with the salt-breeding *A. subpictus* larvæ. They were also compared with fresh-water *A. subpictus* collected from various other places during the survey work.

For the purpose of studying the chaetotaxy (i) last larval moults of both the species were compared in large numbers and results confirmed in every case by the corresponding hatched out adults, (ii) living mature larvæ (3rd and 4th instars) of both the species were compared and the results similarly confirmed by adults eventually reared from them.

It is not the intention of this paper to go into details of larval characters noted by other workers. Only the points that will help others in distinguishing *A. ludlowi* from *A. subpictus* are given here —

I *Colour* —Brown or brownish green

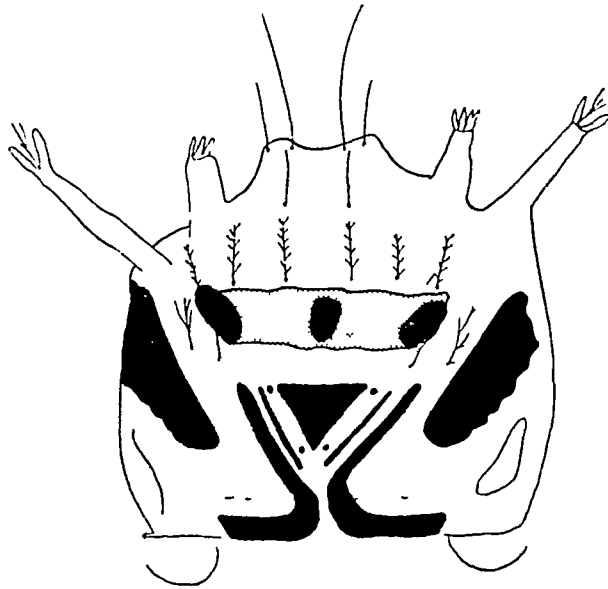


Fig 1 —Head spots of *A. ludlowi*

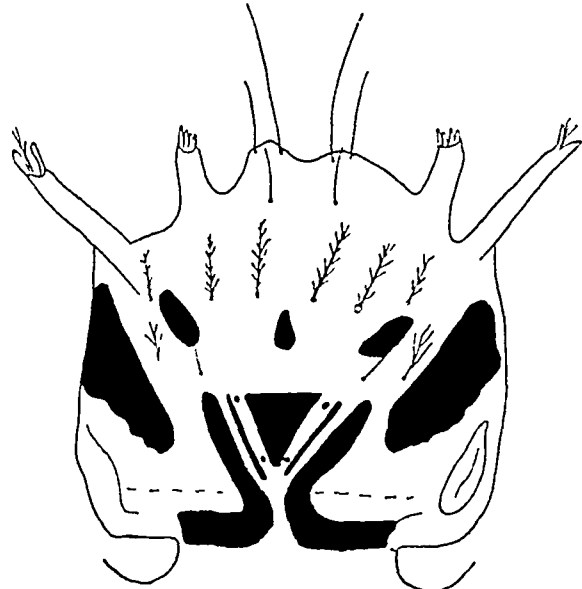


Fig 2 —Head spots of *A. subpictus*

II *Head spots* —The head spots in the mature larvæ (in the 3rd and 4th instars) of *A. ludlowi* and salt-water *A. subpictus* are quite different from that of *A. subpictus* breeding in fresh water. Mature *A. ludlowi* shows a brownish or darkish enveloping cloud surrounding the three dark spots, situated a little behind the middle of the fronto-clypeus more or less like that found in larvæ of *A. fuliginosus* group (vide Fig 1). This is always the case with *A. ludlowi*.

larvæ in their 3rd and 4th instars. In case of *A. subpictus* breeding in salt water a majority of them show a similar well-developed cloud around the spots, only a small number showing a tendency to develop without it.

In case of *A. subpictus*, collected from fresh water, the larvæ have not been found to develop this well-defined band-like cloudiness around these three dark spots (Fig 2), but it is not unusual to find an extensive and deep cloudiness enveloping the posterior half of the fronto-clypeus including all the dark spots of the head from the middle of the fronto-clypeus to the posterior end.

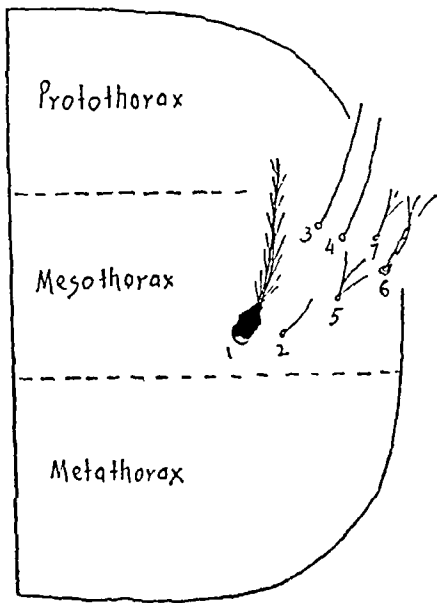


Fig 3—*A. ludlowi*. Most common form of branching of the dorsal hair No 5 (Pur) in the mesothorax

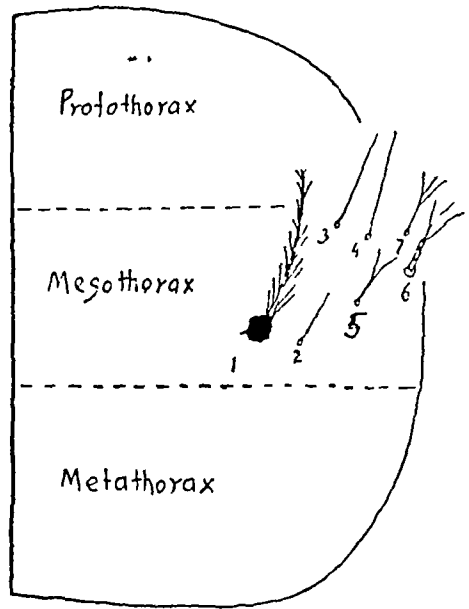


Fig 4—*A. subpictus*. Most common form of branching of the dorsal hair No 5 (Pur) in the mesothorax

III Branching of the dorsal hair No 5 (Pur) on the mesothorax of *A. ludlowi*—*A. ludlowi* larvæ, in their 3rd or 4th instars bred out in the laboratory (1) in fresh water, (2) or in salt water having a similar concentration to that found in their natural breeding places, (3) or when they are collected from their natural breeding places, have been found to have the following mode of branching (Fig 5) —

GROUP I—Most common form 85 per cent of cases (Fig 3) There are three branches on both sides arising towards the base of the hair. Anopheles larva closely resembling *A. subpictus* in every other respect and having this feature along with the head pattern above mentioned never fail to hatch out as *A. ludlowi*.

GROUP II—Four branches on one side and three branches on the other (in 5 per cent of cases) The site of branching is near the base of the hair as in Group I

GROUP III—The dorsal hair No 5 (Puri) in the mesothorax has three branches on one side and two branches on the other (in 10 per cent of cases) The point where the third branch comes off is towards the base

In no full grown larva (3rd and 4th instars) of *A. ludlowi* have we been able to find —

- (1) Two branches only on either sides (most common form in *A. subpictus*)
- (2) A combination of a bifid hair on one side with a simple one on the other (the next commonest form in *A. subpictus*)

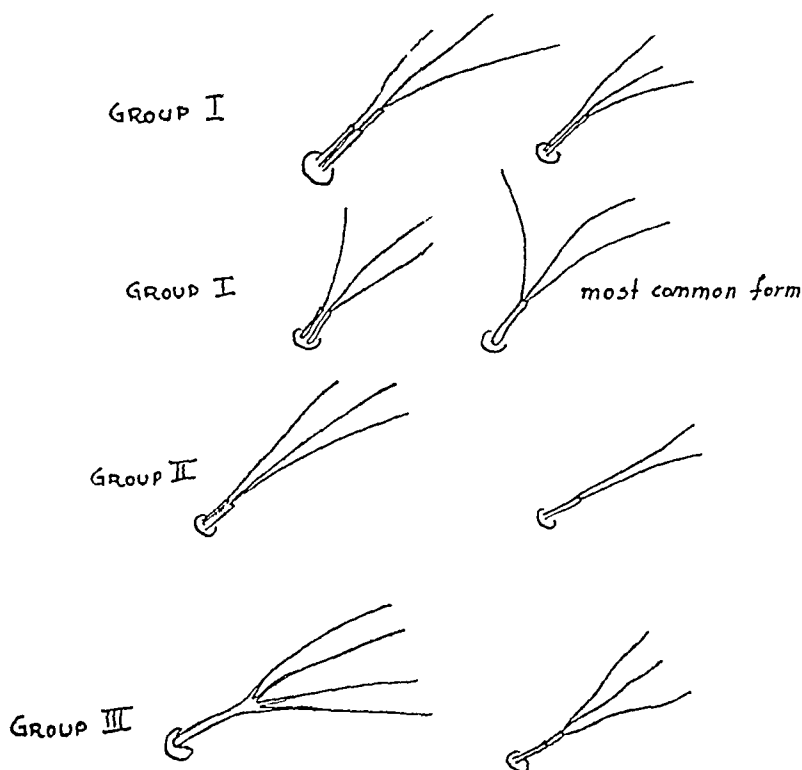


Fig 5—*A. ludlowi* Variation in branching of the dorsal hair No 5 (Puri) of the mesothorax.

Branching of the dorsal hair No 5 (Puri) on the mesothorax in case of *A. subpictus* (Giles)

A mature *subpictus* larva (in the 3rd or 4th instar) when bred out in fresh water, salt water or found in their natural habitat or taken from the salt-water lake has been found to have the following mode of branching of the dorsal hair No 5 (Puri) in the mesothorax (Fig 6)

GROUP I—*Most common form* 80 per cent of cases (Fig 4) *Two branches on either side* They may branch at any place from the base to the apex or be bifid, but the most common form is near the apex

GROUP II—(In 13 per cent of cases) A combination of two branches on one side with a simple one on the other

GROUP III—A combination of two branches on one side with three on the other—in 6 per cent of cases only The third branch usually comes off from a point towards the apex

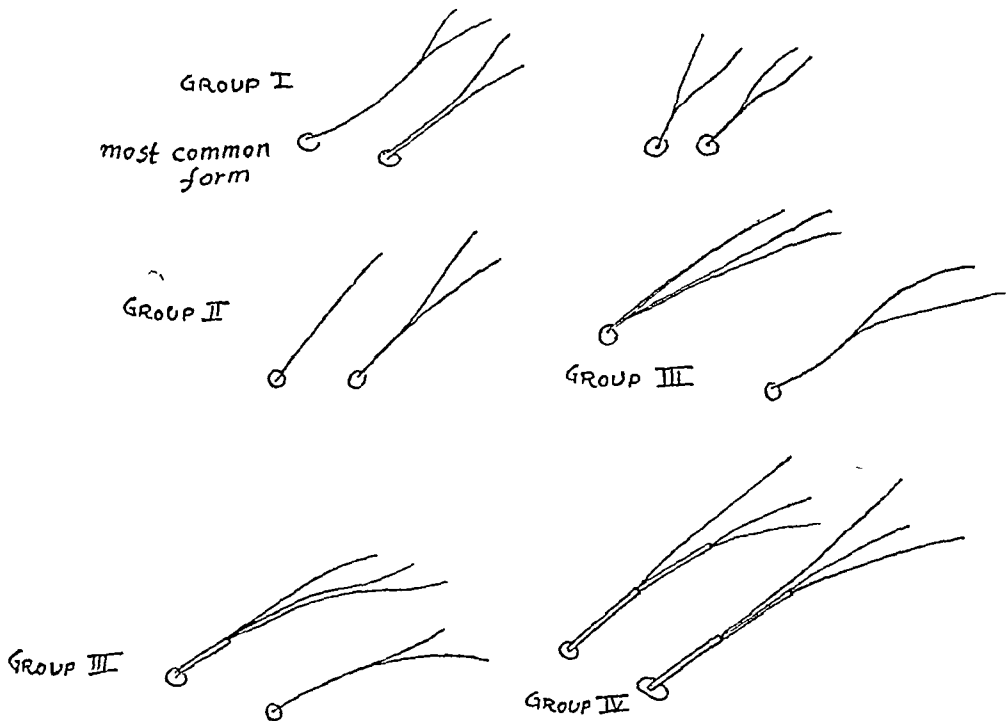


Fig. 6.—*A subpictus* Variation in branching of the dorsal hair No 5 (Pur1) of the mesothorax

GROUP IV—A combination of three branches on both the sides—*only in one per cent of cases*—a very rare form Here again the branching takes place rarely nearer the base than half the length of the hair

From the above comparison of the mode of branching of the 5th dorsal hair (Pur1) in the mesothorax of *A ludlowi* and *A subpictus* it is possible to separate the two species with fair accuracy in 93 per cent of cases

Summary of the procedure to identify *A ludlowi* —

(1) Pick up those larvæ having the general characters of *A subpictus* among the collections with the head spots as described above

(2) Then look for the mode of branching in the dorsal hair No 5 (Puri) of the mesothorax. A larva having the head pattern as described above with the dorsal hair No 5 (Puri) of the mesothorax, having three or more branches on both sides, the branching coming off close to the base, will rarely fail to hatch out as *A. ludlowi*. By this one can detect even a single specimen of *A. ludlowi* larva from amongst a number of salt-breeding *A. subpictus* breeding in common.

Differential identification between *A. ludlowi* and other closely allied species —

(1) *A. tessellatus* var. *punctulata* — This species shows a head marking like that of *A. ludlowi* but the mode of branching (only 2-3 branches) of the internal shoulder hair is a characteristic point to separate it from *A. ludlowi* which has always more than three branches and the number may reach up to 20.

(2) *A. culicifacies* as described by Puri —

(i) The hair No 1 on the metathorax or thoracic palmate hair has numerous lanceolate leaflets without a differentiating filament number varying from 6 to 12.

(ii) The feathered dorsal hair of the anterior pair of the pleural hairs in the prothorax and also the simple ventral hair of the anterior pair of the pleural hairs in the metathorax are sufficient distinguishing features in separating the two species.

(iii) The prominent and pigmented roots of the internal and middle shoulder hairs are also found in *A. ludlowi* and *A. subpictus* breeding in salt water—so this is not a definite point of differentiation on which one can rely with much confidence.

(3) *A. stephensi* — The external occipital hair is either bifid or trifid while in the case of *A. ludlowi* these branches usually exceed three in number.

The author expresses his sincere gratitude to Dr R. B. Khambata, the Director of Public Health, Bengal, and to Dr S. N. Sui, Assistant Director of Public Health, Malania Research, Bengal, for giving him ample facility and encouragement.

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A CONSTANT HUMIDITY APPARATUS FOR MOSQUITOES.

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[Received for publication, December 14, 1931]

IN experimenting on the effect of different humidity concentrations on mosquitoes, the authors have experienced much difficulty in trying to obtain constant humidity conditions and had tried several methods without success. While it is fairly easy to obtain a constant 100 per cent humidity in any closed chamber, it is difficult to maintain a constant humidity condition at concentrations lower than the saturation point. Should one succeed in setting the humidity at a certain level in a chamber at the time of starting the experiment, changes of temperature, such as occur during the course of the day, would produce large variations in the humidity inside the chamber. A further difficulty in regard to keeping live mosquitoes under constant humidity conditions is that it is necessary to keep some exposed water for the mosquitoes to feed on, as otherwise they die within a short time, and any introduction of water alters the humidity that may have been adjusted originally. A constant humidity apparatus for mosquitoes should be such as would provide exposed water for the insects and, at the same time, it should be capable of constantly maintaining the same concentration of humidity at constant temperatures, and even in spite of variations in the atmospheric temperature.

The authors have devised a constant humidity apparatus which fulfils these requirements. They have tested this apparatus under different conditions and have found it to be entirely reliable in maintaining constant humidity of the air in the chamber under conditions of constant temperature as well as at room temperature subject to diurnal variations. In every one of the many tests carried

out, the apparatus maintained a constant humidity, with only a small deviation from the mean, even during tests which extended over several weeks. The relative humidity of the chamber is not affected in any way by diurnal variations of temperature. Even when the difference between the maximum and minimum room temperature during the day was as much as 5°C , the apparatus maintained the same relative humidity throughout the period of test. The apparatus provides a continuous supply of exposed water in the humidity chamber for the mosquitoes which is an important factor as mosquitoes die away quickly if kept without a supply of water. The apparatus is simple, portable, easy to manipulate and it costs less than £2. This portable humidity control apparatus has given entirely satisfactory results and in the present paper, the authors describe the construction of the apparatus and the technique of working it. They also furnish the results of tests carried out to ascertain its reliability as a constant humidity apparatus, and its suitability for keeping live mosquitoes under different constant humidity conditions.

Construction of the apparatus

The apparatus is illustrated in Fig 1. A rectangular museum jar 6" by 8" and 10" high, or a circular jar measuring about 8" in diameter and 10" high, is fitted with a thick glass lid having a bottle neck $1\frac{1}{2}$ " in diameter. The jar should be one of clear white glass free from air bubbles. The edge of the jar and the lid are well ground to ensure air-tight fit. Through the bottle-neck of the lid is fitted a Regnault's dew-point hygrometer consisting of a polished silver test-tube 8" long and $\frac{3}{4}$ " in diameter, fitted with a three-bore India rubber stopper to take two bent-glass tubings and a thermometer. One of these glass tubes is long and nearly touches the bottom of the test-tube, while the other does not reach much below the level of the stopper. The outside ends of the two glass tubes are provided with short lengths of India rubber tubings and their ends are closed with pinch-cocks. The thermometer (Fig 1, T) has a long unmarked stem about 8" long, and should read -10° to $+40^{\circ}$ Centigrade or 10° to 120° Fahrenheit, with divisions to read fractions of a degree accurately. The bulb of the thermometer should be pushed to about half an inch of the bottom of the tube, but should not touch the bottom. The rubber stopper of the silver tube is removed and a quantity of ether sufficient to fill half to three-fourths of the test-tube is poured into it. The rubber stopper is then replaced and the ends A and B of the rubber tubings closed with pinch-cocks. The silver tube goes into a $1\frac{1}{2}$ " India rubber stopper with a bore $\frac{3}{4}$ " in diameter and the whole hygrometer apparatus is thus fitted to the bottle neck in the lid of the glass jar (see Fig 1). The fittings should be as air-tight as possible.

Within the jar (the humidity chamber) are kept the following four articles (1) A glass beaker (4" high and $1\frac{1}{2}$ " in diameter) half filled with concentrated sulphuric acid (Fig 1, C), the mouth of the beaker is wiped dry of the acid and a small piece

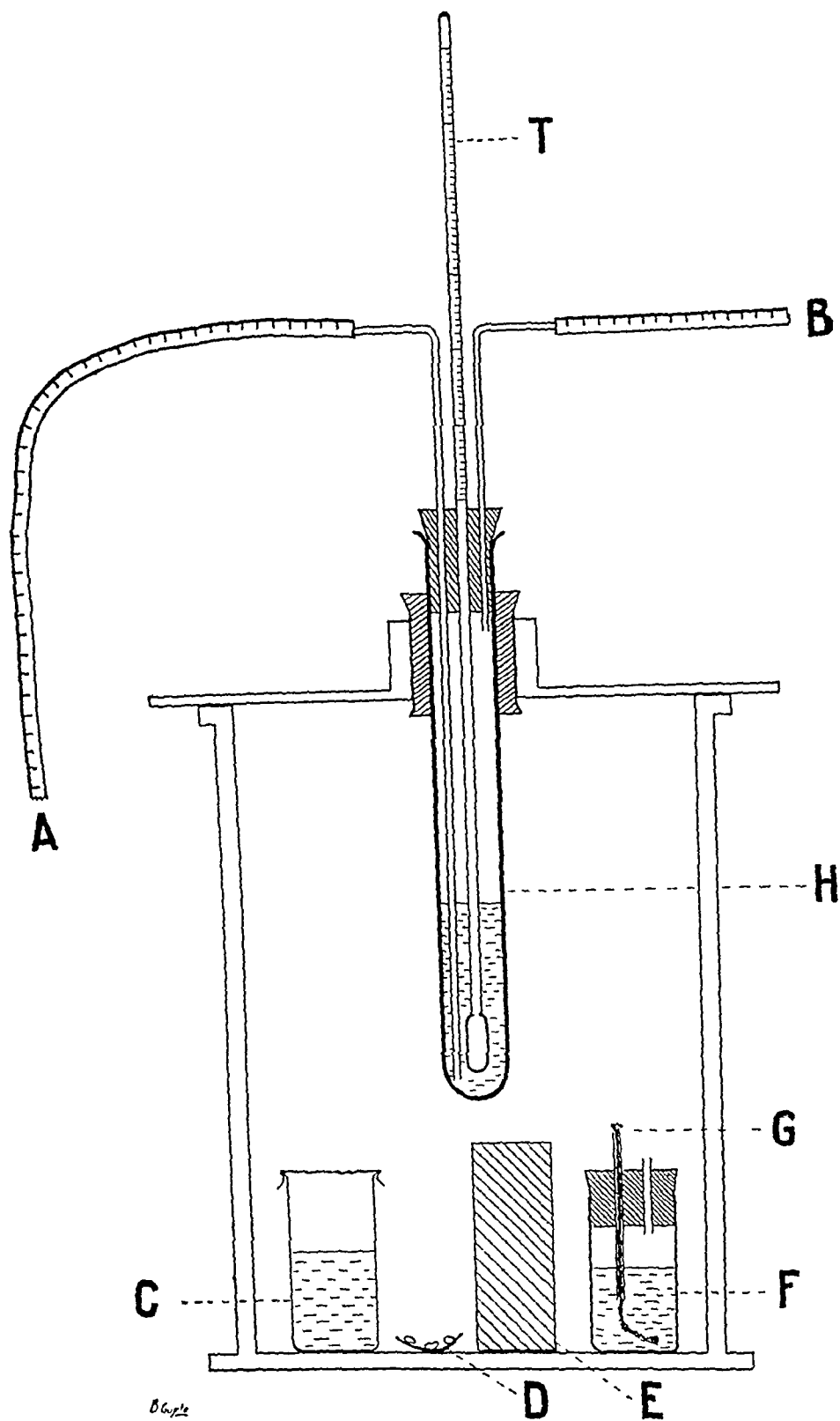


Fig 1

Diagrammatic illustration of the apparatus H—silver-tube hygrometer thermometer T, C—beaker with sulphuric acid, D—watch glass with raisins, E—block of compressed charcoal, F—humidifier, G—wet-wick of humidifier,

of mosquito netting is tied round its mouth. The acid used should be of a high standard of purity, preferably Merck's or Kahlbaum's, less pure and commercial acids may give off fumes which are detrimental to the life of the mosquitoes. (2) A flat bottomed glass specimen tube (about 3" by 1½") fitted with a two-bore India rubber stopper through which pass two glass tubes of a narrow calibre about 3 mm in diameter (Fig 1, F), one of the tubes is long and reaches to within half an inch of the bottom, while the other is short and projects just beyond the stopper both above and below. Through the longer glass tubing passes a clean cotton wick reaching the bottom of the vessel, the wick is cut flush with the top of the glass tube. The vessel is filled with sterile distilled water. This wet wick bottle is here called the 'humidifier'. (3) In addition to the acid container and the humidifier a small block of wood or a block of compressed charcoal (Fig 1, E) is kept in the humidity chamber for mosquitoes to rest on, and (4) A watch glass with a few fresh raisins.

After placing these articles on the floor of the humidity chamber, the edge of the jar is smeared lightly with white vaseline and the lid placed in position and lightly pressed to ensure an air-tight fit. The hygrometer is then inserted into the bottle neck of the lid. Fig 1 is an illustration of the fully fitted up apparatus.

In the humidity chamber of the apparatus which is set in the manner described above, the two processes of (1) evaporation of water by the humidifier and (2) absorption of water vapour by the acid happen simultaneously. After some time, a balance is established between the two processes and a constant humidity condition is brought about within the chamber. It takes a few hours' time for this equilibrium to be established, it is advisable to leave the apparatus overnight and determine the humidity concentration of the air in the chamber on the following morning.

Manipulation

Two data are required for the determination of humidity of the chamber (a) the initial temperature of the chamber and (b) the 'dew-point' of the atmosphere in the humidity chamber. The following procedure is followed.

After noting the initial reading of the thermometer, the rubber stopper of the hygrometer is removed to ascertain if there is sufficient ether in the silver tube. If it is not at least half full with ether, a small amount is added to make up that quantity, and the cork again placed in position. The operator then removes the two pinch-cocks closing the rubber tubes A and B (Fig 1) and blows air from his mouth through A. The air thus blown bubbles through the ether causing it to evaporate and thereby lowering its temperature. The blowing of air should not be continued for more than a few seconds at a time. After each blowing, the pinch-cocks are replaced in position. The operator then watches the silver tube for any cloudiness on its surface as the result of dew formation. He waits till the temperature continues to fall. If the temperature has again become steady and there is

yet no formation of dew on the silver tube, the process of blowing air through A is repeated in the same manner until dew formation is observed on the silver tube. The amount of air blown through the hygrometer during each subsequent occasion should be gradually diminished so as not to lower the temperature too suddenly and thereby going beyond the dew-point too quickly. The temperature is noted the moment any definite haziness is observed on the silver tube, and no more air is blown thereafter. The rubber tubes A and B are closed with pinch-cocks to prevent further evaporation of ether. The operator watches the silver tube and notes the temperature when the dew disappears on the silver tube. The mean of the two readings gives the 'dew-point'.

The difference in temperature between the formation of dew and the disappearance of dew should be as small as possible in order to obtain the most accurate readings. With practice and careful manipulation it should not be difficult to fix the dew-point with difference of 1°C or 0.5°C between the point of appearance and disappearance of dew or even less than that. The smaller this range, the greater is the accuracy. With very high humidities, however, it takes a longer time for the dew to disappear and the difference in temperature between the appearance and disappearance of dew may often be as much as 3°C .

Having determined the dew-point, the relative humidity figure is obtained by the following formula —

$$\frac{\text{Vapour Pressure at Dew-point}}{\text{Vapour Pressure at Initial Temp}} \times 100 = \text{Relative Humidity}$$

Two tables are furnished at the end of this paper giving the vapour pressure in millimetres of mercury for degrees Centigrade (Table I) and in inches of mercury for degrees Fahrenheit (Table II), abstracted from Smithsonian Meteorological Tables, 1893 (*see* pages 1106 and 1109).

The humidity concentration in the chamber is determined by two factors, the evaporating capacity of the humidifier and the absorptive capacity of the acid. If it is found necessary to have a higher or a lower humidity than that obtained in the particular instance, the lid of the chamber is removed and the humidifier wick is adjusted afresh. If a lower humidity is required, the wick is pushed in slightly, if a higher humidity is required it is made to project out. The same result can be achieved by increasing or decreasing the diameter of the beaker containing the acid. It is advisable, however, not to change both but to keep one of them, preferably the acid container, constant. By such manipulation and testing of the apparatus once or twice one can easily get the required humidity. The strength of the acid should be varied in accordance with the humidity required. For relative humidities between 10 and 40 strong concentrated sulphuric acid should be used. For relative humidities 40 to 70, a mixture of equal parts of sulphuric acid and water should be used, and for humidities between 70 to 90, an acid mixture consisting of 3 parts of water to 1 of strong sulphuric acid is recommended. Within each of the three

ranges mentioned above any particular humidity concentration can be obtained by an adjustment of the wick of the humidifier. Once these are adjusted and tested with any particular apparatus and the operator is familiar with these adjustments, he should be able to guess at the humidity concentration that would result from any particular combination of these factors. For very low humidities as for example, below 20 per cent it would be found convenient to have two acid containers instead of one. For very high humidities, as for example above 85 per cent, two humidifiers may be used with advantage instead of one.

In the case of humidities above 75 per cent, raisins kept in the humidity chamber as food for the mosquitoes are liable to get mouldy. In view of this difficulty the authors use an additional humidifier containing a 3 per cent glucose solution. When this is done, there is no necessity for keeping raisins inside the humidity chamber.

It is advisable to run the apparatus for 12 hours or longer after setting it and to make a few preliminary humidity determinations to see if the required humidity is available by the particular adjustment of the acid container and the humidifier. If the required humidity is not available fresh adjustments are made until the required humidity is obtained.

When the apparatus has thus been set to the particular humidity required, the experimental mosquitoes are let into the chamber by means of test-tubes through the bottle-neck of the lid after removing the hygrometer. When the mosquitoes have been let in, the hygrometer is replaced in position. The experimental mosquitoes in the humidity chamber have access to water (from the wick of the humidifier) and food (raisins or glucose solution) and they live in an atmosphere having the same concentration of humidity throughout the period. Mosquitoes can be kept in these chambers for many days continuously and even for several weeks under constant humidity conditions.

At the close of the experiment, the mosquitoes which were kept in the humidity chamber will have to be collected from inside the apparatus. The hygrometer is removed from the bottle-neck of the glass lid and the cavity plugged with some cotton-wool. The apparatus is then placed under a mosquito cage as illustrated in Fig 2. The cage consists of a rectangular wooden frame with a glass pane fitting into a groove on top and a loose cloth bag hanging from it, the front side of the bag comes out as a long sleeve for inserting the hand for catching the mosquitoes. The cloth bag has a longitudinal slit below and the edge of this slit has a length of elastic tape sewn on to it. The humidity apparatus from which the hygrometer has been removed is now placed under this cage and the mouth of the jar is pushed into the cloth bag through the slit at bottom. The operator thrusts his hand through the sleeve, removes the glass lid of the humidity chamber and places it to one side in the cage. The mosquitoes resting inside the jar are then disturbed and made to fly into the cage and caught in test-tubes. Great care should be taken in

handling the apparatus lest by any chance the acid kept in the jar is spilt with severe consequence

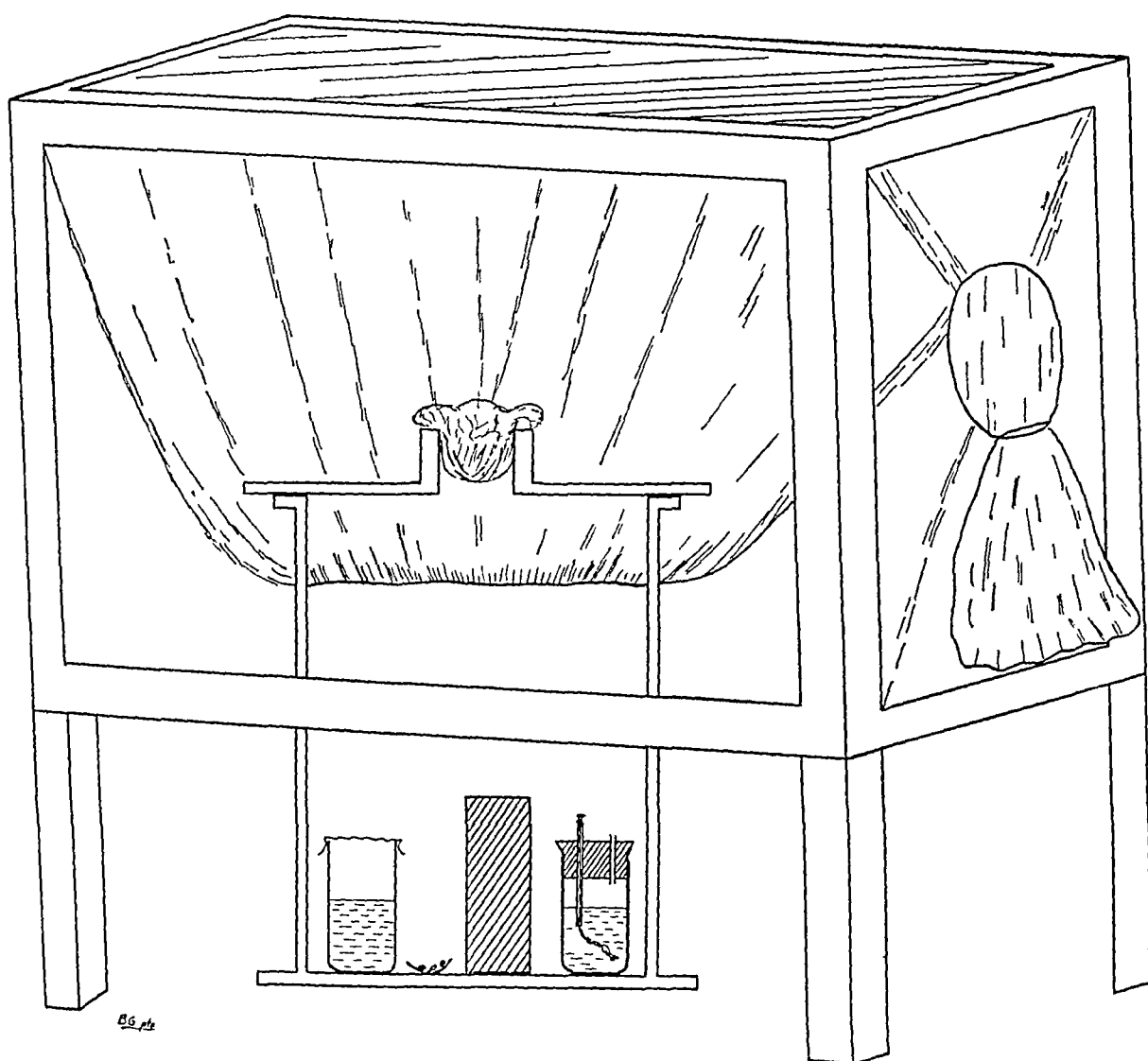


Fig 2

Diagram to illustrate arrangement for re-collecting mosquitoes from inside the humidity chamber

Tests on reliability

The humidity control device described in this paper has been put to a thorough test both under conditions of constant temperature and under conditions subject to diurnal variations of temperature. Under conditions of constant temperature, the relative humidity of the atmosphere in the chamber was observed to be constant even for prolonged periods of 10 to 15 days. The maximum variation from

the mean has been just about 1 and never more than 2 units from the mean relative humidity. Even under conditions in which the temperature varied during the course of the day the apparatus has been observed to maintain constant humidity all the time, with only a small deviation from the mean, usually between 1 and 2, rarely more, but never more than 3 units.

Out of a large number of tests carried out on the reliability of the humidity control apparatus for maintaining constant humidity under diurnal conditions of temperature, a few are here cited as illustrations. The records of experiments A to I given below are the results of tests carried out to determine the efficiency of the apparatus at different degrees of humidity, from 10 per cent to 90 per cent. In each of the different grades of humidity the apparatus was found to be reliable in maintaining a constant humidity condition with only a small variation from the mean.

In all these experimental tests the apparatus was set on the first day in the afternoon and some time (at least 12 hours) was allowed to elapse before commencing observations on the humidity of the atmosphere in the chamber.

EXPERIMENT A

	Time	Initial temperature, °C	Dew point, °C	Relative humidity per cent
2nd Day	6 A M	27.0	-2.0	14.9
" "	10 A M	29.5	0.0	14.9
" "	2 P M	30.2	1.2	15.6
3rd Day	6 A M	27.2	-1.0	15.9
" "	11 A M	29.0	-1.0	14.3
" "	3 P M	30.6	0.2	14.2
5th Day	9 A M	28.2	-2.2	13.7
" "	1 P M	30.0	0.0	14.5

Range of temperature during test 27.0°C to 30.6°C

Average relative humidity 14.8

Maximum deviation from mean humidity ± 1.1

In this test, the apparatus maintained the low humidity of 15 per cent constantly and the maximum deviation from the mean was only 1.1.

EXPERIMENT B

	Time	Initial temperature, °C	Dew point, °C	Relative humidity, per cent
2nd Day	6 A M	27.6	5.6	24.7
" "	10 A M	28.2	6.1	24.7
" "	2 P M	29.0	7.0	25.1
3rd Day	6 A M	27.2	6.0	26.0
" "	1 P M	28.6	6.5	24.8

Range of temperature during test 27.2°C to 29.0°C .

Average relative humidity 25.1

Maximum deviation from mean humidity ± 0.9

The relative humidity in the humidity chamber was constant at 25 per cent with a maximum deviation from mean of only ± 0.9 humidity

EXPERIMENT C

	Time	Initial temperature, °C	Dew point, °C	Relative humidity, per cent
2nd Day	7 A M	29.4	13.4	37.5
" "	2 P M	33.0	15.0	33.9
" "	5 P M	31.0	14.0	35.6
3rd Day	6 A M	28.3	11.8	38.1
" "	11 A M	30.2	13.1	35.2
" "	3 P M	27.6	11.2	36.1

Range of temperature during test 27.6°C to 33.0°C

Average relative humidity 35.7

Maximum deviation from mean humidity ± 1.8

In this experiment in which the relative humidity was set at about 36 per cent the apparatus was found to maintain a constant humidity with a maximum variation of only ± 1.8 although the temperature varied through 5.4°C

EXPERIMENT D

	Time	Initial temperature, °C	Dew point, °C	Relative humidity per cent
2nd Day	6 A M	27.0	12.0	39.4
" "	10 A M	28.5	13.7	40.5
" "	2 P M	30.2	15.0	39.8
3rd Day	6 A M	26.4	12.1	41.1
" "	10 A M	28.6	13.0	38.3
" "	1 P M	29.4	13.5	37.8

Range of temperature during test 26.4°C to 30.2°C

Average relative humidity 39.5

Maximum deviation from mean humidity ± 1.7

In this test the apparatus was set for a humidity concentration of 40 per cent humidity, and it maintained a constant humidity with a maximum deviation of only 1.7 from the mean, in spite of a diurnal range of temperature of 3.8°C

EXPERIMENT E

	Time	Initial temperature, °C	Dew-point, °C	Relative humidity, per cent
2nd Day	7 A M	27.6	15.8	48.7
" "	10 A M	29.0	16.4	46.6
" "	3 P M	30.0	17.5	47.2
3rd Day	7 A M	27.0	15.1	48.2
" "	10 A M	29.0	16.8	47.8
" "	3 P M	28.2	16.2	49.2

Range of temperature during test 27.0°C to 30.0°C

Average relative humidity 47.8

Maximum deviation from mean humidity ± 1.2

In this test, the apparatus which was set at about 48 per cent humidity maintained a constant humidity with a small deviation of 1.2 from the mean

EXPERIMENT F

	Time	Initial temperature, °C	Dew point, °C	Relative humidity, per cent
2nd Day	6 A M	27.0	17.8	57.2
" "	10 A M	28.5	19.0	56.5
" "	2 P M	30.5	21.0	57.0
3rd Day	6 A M	26.8	18.0	58.6
" "	10 A M	28.3	19.0	57.2
" "	1 P M	29.3	20.5	59.2

Range of temperature during test 26.8°C to 30.5°C

Average relative humidity 57.6

Maximum deviation from mean humidity ± 1.6

In this experiment the apparatus maintained a constant humidity of 58 per cent with a small deviation of 1.6 from mean

EXPERIMENT G

	Time	Initial temperature, °C	Dew-point, °C	Relative humidity, per cent
2nd Day	7 A M	29.6	24.4	73.7
" "	2 P M	33.0	27.0	70.8
" "	5 P M	31.0	25.0	70.5
3rd Day	6 A M	28.6	23.2	72.6
" "	11 A M	30.2	24.0	69.5
" "	3 P M	28.2	22.8	72.6

Range of temperature during test 28 2°C to 33 0°C

Average relative humidity 71 6

Maximum deviation from mean humidity $\pm 2 1$

The apparatus which was set at about 70 per cent humidity maintained a constant humidity in spite of a variation of temperature amounting to 4 8°C during the period of observation. The deviation from normal in the humidity value was 2 1.

EXPERIMENT H

	Time	Initial temperature, °C	Dew-point, °C	Relative humidity, per cent
2nd Day	6 A M	27 8	24 5	82 3
" "	10 A M	28 5	25 0	81 4
" "	2 P M	30 0	26 2	80 2
3rd Day	10 A M	27 2	23 4	79 8
" "	1 P M	29 0	25 0	79 1

Range of temperature during test 27 2°C to 30 0°C

Average relative humidity 80 6

Maximum deviation from mean humidity $\pm 1 7$

This experiment set for a humidity of about 80 per cent maintained a constant humidity continuously with a small variation of only 1 7

EXPERIMENT I

	Time	Initial temperature, °C	Dew-point, °C	Relative humidity, per cent
2nd Day	6 A M	28 1	26 2	89 5
" "	10 A M	28 4	26 5	89 4
" "	2 P M	29 8	27 0	85 0
3rd Day	6 A M	27 4	25 2	87 8
" "	1 P M	28 8	26 5	87 4

Range of temperature during test 27 4°C to 29 8°C

Average relative humidity 87 8

Maximum deviation from mean humidity $\pm 2 8$

In this experiment a very high humidity of 88 per cent was maintained constantly and the maximum deviation from the mean for the period of observation was comparatively small, namely, 2 8

The results of these tests prove conclusively that the constant humidity apparatus devised by the authors is reliable and obtains constant humidity conditions either at constant temperatures or under conditions subject to the

ordinary diurnal fluctuations of temperature. In each of the different grades of humidity from 10 to 90 per cent the apparatus has been observed to work efficiently and to maintain a constant humidity with only a small deviation which was generally less than ± 2 from the mean.

The tests detailed above (Experiments A to I) were run for three days each. Owing to lack of time these observations could not be continued for longer periods. In a few tests carried out over prolonged periods the apparatus was observed to maintain constant humidity for two weeks or even more. One of these tests (Experiment L) which was run for 18 days continuously discussed in a later part of this paper shows that the apparatus works efficiently even during prolonged observations.

Tests under temperate conditions

The above observations were carried out at room temperatures in a tropical country and it was desired to test the apparatus for its workability under temperate conditions. For this purpose the apparatus was set in the cool room of the Calcutta School of Tropical Medicine (through the courtesy of the Director, Colonel H. W. Acton, I.M.S.) and observations made under temperate conditions. The following two experiments show that the apparatus is reliable even under temperate conditions.

EXPERIMENT J

	Time	Initial temperature, °C	Dew point, °C	Relative humidity, per cent
2nd Day	3 30 P M	20 0	19 0	94 0
3rd "	1 P M	20 0	19 0	94 0
5th "	4 P M	20 3	19 0	92 3

Range of temperature during test 20 0°C to 20 3°C

Average relative humidity 93 4

Maximum deviation from mean humidity $\pm 1 1$

EXPERIMENT K

	Time	Initial temperature, °C	Dew point, °C	Relative humidity, per cent
2nd Day	3-30 P M	18 0	-5 0	20 6
3rd "	1 P M	18 5	-6 0	18 5
5th "	3 P M	19 0	-6 0	18 0
6th "	3 P M	18 5	-6 5	17 8
8th "	11-30 A M	20 0	-5 5	17 5
9th "	2-45 P M	21 0	-5 0	17 1

Range of temperature during test 18 0°C to 21 0°C

Average relative humidity 18 3

Maximum deviation from mean humidity ± 2.3

These two experiments, one with a high humidity and the other with a low humidity, show that even under temperate conditions this humidity control apparatus is capable of maintaining a constant humidity with only a small deviation from the mean humidity value

Suitability for keeping live mosquitoes

It may be apprehended by some that although this apparatus is capable of maintaining constant humidity, it may not be good enough for keeping mosquitoes alive in it owing to lack of sufficient aeration within the humidity chamber. To determine whether conditions inside the closed humidity chamber were in any way adverse to the life of the mosquitoes, experiments were conducted with the apparatus after letting live mosquitoes into the humidity chamber. Several such trials with this apparatus have shown that the amount of air available inside the chamber is sufficient to keep mosquitoes alive for many days at a stretch, and even for as long a period as 18 days as was observed in one experiment (Experiment L) the record of which is given below —

EXPERIMENT L

Apparatus set in the afternoon of the 1st day and mosquitoes let into the humidity chamber

	Time	Initial temperature, °C	Dew point, °C	Relative humidity, per cent
2nd Day	3 P M	29.4	26.3	83.5
3rd "	6 A M	27.8	24.0	79.9
" "	1 P M	29.0	25.5	81.5
4th Day	6 A M	27.0	23.8	82.7
" "	1 P M	27.5	23.8	80.3
5th "	6 A M	27.0	23.3	80.2
" "	1 P M	27.7	24.0	80.3
6th "	7 A M	27.8	24.4	81.8
7th "	7 A M	27.5	24.3	82.7
8th "	7 A M	28.1	25.0	83.3
" "	1 P M	29.4	26.0	82.0
9th "	7 A M	27.2	23.8	81.8
" "	1 P M	27.0	23.9	83.2
10th "	7 A M	25.7	22.8	84.1
" "	1 P M	25.8	22.5	82.1

TABLE I*

Pressure of aqueous vapour

(Broch)

Metric Measures

—15° to +45° Centigrade, in millimetres of mercury

Temperature	0 0°	0 1°	0 2°	0 3°	0 4°	0 5°	0 6°	0 7°	0 8°	0 9°
C	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
—15°	1 44	1 43	1 42	1 40	1 39	1 38	1 37	1 36	1 35	1 34
14	1 56	1 55	1 54	1 52	1 51	1 50	1 49	1 48	1 46	1 45
13	1 69	1 68	1 67	1 65	1 64	1 63	1 61	1 60	1 59	1 57
12	1 84	1 82	1 81	1 79	1 78	1 76	1 75	1 74	1 72	1 71
11	1 99	1 97	1 96	1 94	1 93	1 91	1 90	1 88	1 87	1 85
—10°	2 15	2 13	2 12	2 10	2 09	2 07	2 05	2 04	2 02	2 00
9	2 33	2 31	2 29	2 27	2 26	2 24	2 22	2 20	2 19	2 17
8	2 51	2 50	2 48	2 46	2 44	2 42	2 40	2 38	2 36	2 34
7	2 72	2 69	2 67	2 65	2 63	2 61	2 59	2 57	2 55	2 53
6	2 93	2 91	2 89	2 86	2 84	2 82	2 80	2 78	2 76	2 74
— 5°	3 16	3 14	3 11	3 09	3 07	3 04	3 02	3 00	2 98	2 95
4	3 41	3 38	3 36	3 33	3 31	3 28	3 26	3 23	3 21	3 18
3	3 67	3 64	3 62	3 59	3 56	3 54	3 51	3 48	3 46	3 43
2	3 95	3 92	3 89	3 86	3 84	3 81	3 78	3 75	3 72	3 70
1	4 25	4 22	4 19	4 16	4 13	4 10	4 07	4 04	4 01	3 98
— 0°	4 57	4 54	4 50	4 47	4 44	4 41	4 37	4 34	4 31	4 28
+ 0°	4 57	4 60	4 64	4 67	4 70	4 74	4 77	4 80	4 81	4 87
1	4 91	4 94	4 98	5 02	5 05	5 09	5 12	5 16	5 20	5 23
2	5 27	5 31	5 35	5 39	5 42	5 46	5 50	5 54	5 58	5 62
3	5 66	5 70	5 74	5 78	5 82	5 86	5 90	5 94	5 99	6 03
4	6 07	6 11	6 15	6 20	6 24	6 28	6 33	6 37	6 42	6 46

* See page 1095

TABLE I—*contd*

Temperature	0 0°	0 1°	0 2°	0 3°	0 4°	0 5°	0 6°	0 7°	0 8°	0 9°
C	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
5	6 51	6 55	6 60	6 64	6 69	6 74	6 78	6 83	6 88	6 92
6	6 97	7 02	7 07	7 12	7 17	7 22	7 26	7 31	7 36	7 42
7	7 47	7 52	7 57	7 62	7 67	7 72	7 78	7 83	7 88	7 94
8	7 99	8 05	8 10	8 15	8 21	8 27	8 32	8 38	8 43	8 49
9	8 55	8 61	8 66	8 72	8 78	8 84	8 90	8 96	9 02	9 08
+10°	9 14	9 20	9 26	9 32	9 39	9 45	9 51	9 58	9 64	9 70
11	9 77	9 83	9 90	9 96	10 03	10 09	10 16	10 23	10 30	10 36
12	10 43	10 50	10 57	10 64	10 71	10 78	10 85	10 92	10 99	11 07
13	11 14	11 21	11 28	11 36	11 43	11 50	11 58	11 66	11 73	11 81
14	11 88	11 96	12 04	12 12	12 19	12 27	12 35	12 43	12 51	12 59
15	12 67	12 76	12 84	12 92	13 00	13 09	13 17	13 25	13 34	13 42
16	13 51	13 60	13 68	13 77	13 86	13 95	14 04	14 12	14 21	14 30
17	14 40	14 49	14 58	14 67	14 76	14 86	14 95	15 04	15 14	15 23
18	15 33	15 43	15 52	15 62	15 72	15 82	15 92	16 02	16 12	16 22
19	16 32	16 42	16 52	16 63	16 73	16 83	16 94	17 04	17 15	17 26
+20°	17 36	17 47	17 58	17 69	17 80	17 91	18 02	18 13	18 24	18 35
21	18 47	18 58	18 69	18 81	18 92	19 04	19 16	19 27	19 39	19 51
22	19 63	19 75	19 87	19 99	20 11	20 24	20 36	20 48	20 61	20 73
23	20 86	20 98	21 11	21 24	21 37	21 50	21 63	21 76	21 89	22 02
24	22 15	22 29	22 42	22 55	22 69	22 83	22 96	23 10	23 24	23 38
25	23 52	23 66	23 80	23 94	24 08	24 23	24 37	24 52	24 66	24 81
26	24 96	25 10	25 25	25 40	25 55	25 70	25 86	26 01	26 16	26 32
27	26 47	26 63	26 78	26 94	27 10	27 26	27 42	27 58	27 74	27 90
28	28 07	28 23	28 39	28 56	28 73	28 89	29 06	29 23	29 40	29 57
29	29 74	29 92	30 09	30 26	30 44	30 62	30 79	30 97	31 15	31 33

TABLE I—*concl'd*

Temperature	0 0°	0 1°	0 2°	0 3°	0 4°	0 5°	0 6°	0 7°	0 8°	0 9°
C.	mm	mm	mm	mm	mm	mm	mm	mm	mm	mm
+30°	31 51	31 69	31 87	32 06	32 24	32 43	32 61	32 80	32 99	33 18
31	33 37	33 56	33 75	33 94	34 14	34 33	34 53	34 72	34 92	35 12
32	35 32	35 52	35 72	35 92	36 13	36 33	36 54	36 74	36 95	37 16
33	37 37	37 58	37 79	38 00	38 22	38 43	38 65	38 87	39 08	39 30
34	39 52	39 74	39 97	40 19	40 41	40 64	40 87	41 09	41 32	41 55
35	41 78	42 02	42 25	42 48	42 72	42 96	43 19	43 43	43 67	43 92
36	44 16	44 40	44 65	44 89	45 14	45 39	45 64	45 89	46 14	46 39
37	46 65	46 90	47 16	47 42	47 68	47 94	48 20	48 46	48 73	48 99
38	49 26	49 53	49 80	50 07	50 34	50 61	50 89	51 16	51 44	51 72
39	52 00	52 28	52 56	52 84	53 13	53 41	53 70	53 99	54 28	54 57
+40°	54 87	55 16	55 46	55 75	56 05	56 35	56 65	56 95	57 26	57 56
41	57 87	58 18	58 49	58 80	59 11	59 43	59 74	60 06	60 38	60 70
42	61 02	61 34	61 66	61 99	62 32	62 65	62 98	63 31	63 64	63 97
43	64 31	64 65	64 99	65 33	65 67	66 01	66 36	66 71	67 05	67 41
44	67 76	68 11	68 47	68 82	69 18	69 54	69 90	70 26	70 63	70 99
45	71 36	71 73	72 10	72 48	72 85	73 23	73 60	73 98	74 36	74 75

TABLE II*

Pressure of aqueous vapour

(Broch)

English Measure

0° to 115° Fahrenheit, in inches of mercury

Temperature	0 0°	0 2°	0 4°	0 6°	0 8°
F	Inch	Inch	Inch	Inch	Inch
+ 0 0°	0 0419	0 0454	0 0458	0 0462	0 0467
+ 1 0°	0471	0475	0480	0484	0489
2 0°	0493	0498	0502	0507	0512
3 0°	0517	0522	0526	0531	0536
4 0°	0541	0546	0551	0556	0561
5 0°	0567	0572	0577	0582	0588
6 0°	0593	0598	0604	0609	0615
7 0°	0620	0626	0632	0637	0643
8 0°	0649	0655	0661	0667	0673
9 0°	0679	0685	0691	0697	0704
+ 10 0°	0710	0716	0723	0729	0736
11 0°	0742	0749	0756	0762	0769
12 0°	0776	0783	0790	0797	0804
13 0°	0811	0818	0825	0832	0840
14 0°	0847	0854	0862	0869	0877

* See page 1095

TABLE II—*contd*

Temperature	0 0°	0 2°	0 4°	0 6°	0 8°
F	Inch	Inch	Inch	Inch	Inch
15 0°	0 0885	0 0892	0 0900	0 0908	0 0916
16 0°	0924	0932	0940	0948	0956
17 0°	0965	0973	0981	0990	0999
18 0°	1007	1016	1024	1033	1042
19 0°	1051	1060	1069	1078	1087
+ 20 0°	1097	1106	1115	1125	1134
21 0°	1144	1154	1163	1173	1183
22 0°	1193	1203	1213	1223	1234
23 0°	1244	1255	1265	1276	1287
24 0°	1297	1308	1319	1330	1341
25 0°	1352	1364	1375	1386	1398
26 0°	1409	1421	1433	1445	1457
27 0°	1469	1481	1493	1505	1518
28 0°	1530	1543	1555	1568	1581
29 0°	1594	1607	1620	1633	1646
+ 30 0°	1660	1673	1687	1700	1714
31 0°	1728	1742	1756	1770	1784
32 0°	1799	1813	1828	1842	1857
33 0°	1872	1887	1902	1917	1933
34 0°	1948	1964	1979	1995	2011

TABLE II--*contd*

Temperature	0 0°	0 2°	0 4°	0 6°	0 8°
F	Inch	Inch	Inch	Inch	Inch
35 0°	0 2027	0 2043	0 2059	0 2076	0 2092
36 0°	2109	2125	2142	2159	2176
37 0°	2193	2210	2228	2245	2263
38 0°	2281	2298	2316	2334	2353
39 0°	2371	2390	2408	2427	2446
+ 40 0°	2465	2484	2503	2523	2542
41 0°	2562	2582	2601	2621	2642
42 0°	2662	2683	2703	2724	2745
43 0°	2766	2787	2808	2830	2851
44 0°	2873	2895	2917	2939	2962
45 0°	2984	3007	3030	3053	3076
46 0°	3099	3123	3146	3170	3194
47 0°	3218	3242	3267	3291	3316
48 0°	3341	3366	3391	3416	3442
49 0°	3467	3493	3519	3546	3572
+ 50 0°	3598	3625	3652	3679	3706
51 0°	3734	3761	3789	3817	3845
52 0°	3874	3902	3931	3960	3989
53 0°	4018	4048	4077	4107	4137
54 0°	4168	4198	4229	4259	4290
55 0°	4322	4353	4385	4417	4449
56 0°	4481	4513	4546	4579	4612
57 0°	4645	4679	4712	4746	4780
58 0°	4815	4849	4884	4919	4954
59 0°	4990	5025	5061	5097	5134

TABLE II—*contd*

Temperature	0 0°	0 2°	0 4°	0 6°	0 8°
F	Inch	Inch	Inch	Inch	Inch
+ 60 0°	0 5170	0 5207	0 5244	0 5282	0 5319
61 0°	5357	5395	5433	5471	5510
62 0°	5549	5588	5628	5667	5707
63 0°	5748	5788	5829	5870	5911
64 0°	5952	5994	6036	6078	6120
65 0°	6163	6206	6249	6293	6337
66 0°	6381	6425	6470	6519	6560
67 0°	6605	6651	6697	6743	6789
68 0°	6836	6883	6930	6978	7026
69 0°	7074	7123	7172	7221	7270
+ 70 0°	7320	7370	7420	7471	7522
71 0°	7573	7625	7676	7628	7781
72 0°	7831	7887	7940	7994	8048
73 0°	8102	8157	8212	8267	8323
74 0°	8379	8435	8492	8549	8606
75 0°	8664	8722	8780	8839	8898
76 0°	8957	9017	9077	9137	9198
77 0°	9259	9321	9383	9445	9507
78 0°	9570	9633	9697	9761	9825
79 0°	9890	9955	1 0021	1 0087	1 0153
+ 80 0°	1 0219	1 0286	0354	0422	0490
81 0°	0558	0627	0697	0767	0837
82 0°	0907	0978	1050	1121	1194
83 0°	1266	1339	1413	1487	1561
84 0°	1635	1710	1786	1862	1938

TABLE II—*contd*

Temperature	0 0°	0 2°	0 4°	0 6°	0 8°
F	Inch	Inch	Inch	Inch	Inch
85 0°	0 2015	0 2092	0 2170	0 2248	0 2327
86 0°	2406	2485	2565	2645	2726
87 0°	2807	2889	2971	3054	3137
88 0°	3220	3304	3388	3473	3558
89 0°	3644	3731	3818	3905	3993
+ 90 0°	1 4081	1 4170	1 4259	1 4349	1 4439
91 0°	4530	4621	4712	4805	4897
92 0°	4990	5084	5178	5273	5368
93 0°	5464	5560	5657	5755	5853
94 0°	5951	6050	6149	6249	6350
95 0°	6451	6552	6655	6758	6861
96 0°	6964	7069	7174	7279	7385
97 0°	7492	7599	7707	7815	7924
98 0°	8034	8144	8254	8366	8477
99 0°	8590	8703	8817	8931	9046
+ 100 0°	9161	9277	9394	9511	9629
101 0°	9747	9867	9986	2 0107	2 0228
102 0°	2 0349	2 0471	2 0594	0718	0842
103 0°	0967	1092	1218	1345	1473
104 0°	1601	1730	1859	1989	2120
105 0°	2251	2384	2516	2650	2784
106 0°	2919	3054	3190	3327	3465
107 0°	3603	3742	3882	4023	4164
108 0°	4306	4449	4592	4736	4881
109 0°	5026	5172	5319	5467	5616

TABLE II—*concl'd*

Temperature	0 0°	0 2°	0 4°	0 6°	0 8°
F	Inch	Inch	Inch	Inch	Inch
+ 110 0°	0 5765	0 5915	0 6066	0 6217	0 6369
111 0°	6522	6676	6831	6986	7142
112 0°	7299	7457	7616	7775	7935
113 0°	8096	8257	8420	8583	8747
114 0°	8912	9078	9244	9412	9580
115 0°	8749	9919	3 0089	3 0261	3 0433

ON THE ELECTRIC CHARGE OF ERYTHROCYTES

Part I.

EFFECT OF pH AND QUININE BIHYDROCHLORIDE

BY

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Indigenous Drugs Inquiry, I R F A Series No 30

[Received for publication, December 18, 1931]

INTRODUCTION

THE study of the electric charge of mammalian erythrocytes, bacteria and protozoa forms an interesting branch of biochemistry in view of its relation to hæmolysis(1, 2 & 3), agglutination, immunity and other biological processes(4, 5, 6, 7, 8, 9, 10, 11 & 12) This property also appears to have some bearing on the toxicity of alkaloids towards bacteria, protozoa, etc (13) The earlier investigators have mainly concerned themselves with the determination of the iso-electric point of the corpuscles or bacteria, and the variation of the charge with acidity and correlating these results with those of agglutination In view of the improved technique recently made available on the measurement of cataphoretic velocities by the microscopic method, through the work of Freundlich and Abramson(14), it was considered desirable to study this property more closely in relation to the relevant biological processes The object of this series of papers, of which the present one forms the preliminary part, is to measure the cataphoretic velocity of erythrocytes, protozoa, or bacteria under different conditions and to correlate them with biological processes in the light of the modern theories of colloid chemistry

METHODS

The apparatus used by us is essentially the same as that of Northrop(7) as modified by Freundlich and Abramson (*loc cit*), only the main cell can be detached from the electrode vessels (with a three-way stop-cock). This modification enables one to clean the cell and the electrode vessels much more thoroughly and with greater ease. The dimensions of the particular cell used were 7.5 cm, 0.95 cm and 0.09 cm *.

Suspensions of human red cells were prepared in normal (0.85 per cent) saline solution. To 1 c.c. of blood, 0.1 c.c. of 2 per cent potassium oxalate solution was added. The cells were then centrifuged and washed 6 times with 0.85 per cent saline solution. Experiments were carried out with 1 per cent suspensions. Red cell suspensions were never used after two days from the day of preparation. Preliminary experiments were carried out that showed that the cataphoretic velocity does not change with this procedure (Table I). The suspensions were always kept

* The following scheme of calculating the potential gradient is more convenient and perhaps also more accurate than that suggested by Northrop(7) or Brown and Boom(15). It follows from Ohm's law that E , the drop in potential per cm. in the cataphoresis cell, is

$$E = \frac{c}{\Delta q} \quad (1)$$

where c is the current, Δ the specific conductivity of the suspension and q the cross section of the cell. This procedure also ensures that the conductance of the suspension has not undergone any change due to some accidental causes such as heating effect or migration of ions from the electrode vessels into the cell or vice versa, as any such change would be easily detected by a concomitant change in the current strength. It is also very important to ensure that the cell is of uniform cross section all along its length, for the value of the potential gradient depends on this magnitude and hence the modification of the Northrop Kunitz(16) micro cataphoresis cell (made of pyrex or jena glass drawn out sideways but *not of uniform cross section*) as later on suggested by Abramson(17) is not desirable. To avoid this error, Abramson suggests that hairs should be crossed and cemented on to the top of the cell with Canada balsam and that by taking the point of crossing of the hairs as a reference mark, measurements could be taken always at the same point in the cell. This is perhaps also the reason why he defines q as the cross section of the cell *at the point of measurement (loc cit)*. Evidently it is overlooked that the corpuscles would move through a certain distance and it is not at all certain that q would retain the same value throughout this distance. From these considerations it is evident that the modified apparatus suggested by Freundlich and Abramson (*loc cit*) is to be preferred. The electrodes used were non polarizable and of the type Cu-CuSO₄-agar in isotonic saline solutions. Readings were taken at least three times first and the current being reversed they were repeated again. The microscope was provided with a 28 \times ocular fitted with micrometer and a 20 \times achromatic objective. This magnification was sufficient for our purpose.

The cataphoretic velocity can be calculated from Smoluchowski's equation

$$V = \frac{3}{4} V_{\frac{1}{4}} + \frac{1}{4} V_{\frac{1}{2}} \quad (2)$$

where $V_{\frac{1}{4}}$ and $V_{\frac{1}{2}}$ are the observed velocities of the corpuscles at $\frac{1}{4}$ th and $\frac{1}{2}$ height of the cell. It is more accurate to measure the velocity at different heights and extrapolating the values of $V_{\frac{1}{4}}$ and $V_{\frac{1}{2}}$ from a curve obtained from cataphoretic velocities at different heights and to insert them in equation(2). But for our present purpose, where proteins and substances of similar nature are absent (Northrop, *loc cit*), measurements of the velocities at $\frac{1}{4}$ th and $\frac{1}{2}$ height of the chamber are sufficiently accurate.

in jena glass test-tubes in the cool room, when not in use The results of these preliminary experiments are given in Table I where V denotes the cataphoretic velocity of the red cells in cm per sec per volt per cm

TABLE I

Effect of ageing on the charge of 1 per cent suspension of red cells

	$V \times 10^5$
1 Fresh suspension	11.1
24 hours old	10.9
48 " "	11.5
2 Fresh suspension	9.7
24 hours old	10.1
48 " "	9.9

These experiments show that the negative charge of human red cells are not changed on keeping in the cool room for about 48 hours—a result similar in nature to that already obtained by Freundlich and Abramson with suspensions of horse red blood corpuscles Next, experiments were carried with suspensions obtained from the blood of different normal individuals and the results are given in Table II where V signifies as before

TABLE II

The variation of the charge of red blood cells from different individuals

	$V \times 10^5$		$V \times 10^5$
1	11.1	6	11.17
2	12.7	7	12.9
3	11.3	8	12.05
4	12.5	9	11.5
5	13.1	10	12.3

It appears, therefore, that red cells obtained from the blood of different individuals apparently normal do not possess the same amount of negative charge and

therefore, in our subsequent experiments, we restricted ourselves to the same sample of blood drawn from one individual in order to study the effect of a particular variable

Effect of pH on the negative charge of R B C

In order to study the effect of hydrogen ion concentration on the negative charge of human red blood cells, the suspensions were mixed with Sorensen buffers of different pH values such that the final concentration of the suspension in the mixture becomes 1 per cent. The results are given in Table III and have been obtained from the same sample of blood drawn from the same individual. Experiments were carried out within half an hour of the mixing the suspension with the buffer.

TABLE III

Effect of pH on the charge of R B C suspended in normal saline

pH value	$V \times 10^6$
5.5	13.6
6.0	14.0
6.5	14.3
7.0	15.4
7.5	15.9
8.0	16.6

These experiments show that with decrease in the hydrogen ion concentration, the negative charge of the erythrocytes increases but very slightly. Christophers (*loc cit*) suspended the red blood cells in isotonic glucose solution and studied the variation of their cataphoretic velocity with hydrogen ion concentration with the addition of hydrochloric acid or caustic soda and as such these results cannot be compared with those obtained by him. They, however, are similar to those obtained by Abramson(17) in more acid solutions with acetate-acetic acid buffers. Red cell suspensions were also prepared in isotonic glucose (Merck's pure dextrose was taken) and the variation (5.8 glucose as suggested by Christophers) of the charge with change in hydrogen ion concentration with buffer has been studied. The results are given in Table IV where pH values indicated are those of the buffer solutions.

TABLE IV

Effect of pH on the charge of R B C suspended in isotonic glucose solution

pH value	V $\times 10^5$
5.5	21.5
6.0	23.9
6.5	27.8
7.0	32.0
7.5	35.3
8.0	37.5

Comparing the results in Table IV with those in Table III we see that the variation of the negative charge with pH values is greater in the case of suspensions, prepared in isotonic glucose solution. These results were also obtained within half an hour of the mixing of the suspension with the buffer.

EFFECT OF QUININE BIHYDROCHLORIDE ON THE CHARGE OF R B C AT DIFFERENT pH VALUES

Next the behaviour of the corpuscles suspended both in normal saline and isotonic sugar solutions in presence of different concentrations of quinine bihydrochloride has been studied. The results are given in Table V, where it will be seen that the variation of the charge with concentration of the alkaloidal salt is greater in isotonic glucose than in normal saline solution. These results were obtained within about 15 minutes of the time of mixing and it will also be seen that with 1 in 1,000 quinine bihydrochloride, there is a time effect on the cataphoretic velocity of R B Cs suspended in normal saline solution. Within 2 hours of mixing of the salt, there was also partial hæmolysis. As we noticed this time effect with 1 in 1,000 dilution, we, however, did not proceed up to this dilution in other cases. The study of this time effect is interesting and is left for the present for future investigation. It was, therefore, thought desirable to study the effect of changing the pH value on the charge of R B C in presence of quinine bihydrochloride in isotonic glucose solutions only. It is to be noted that the mixture was prepared in such a way that the final concentration of R B C was 1 per cent, and that of the salt was the concentration desired.

Usually 10 c.c. of a 5 per cent suspension and 1 c.c. of the salt of 50 times stronger concentration and 39 c.c. of a buffer of definite pH value were mixed. The velocities were all measured within 15 minutes of the time of mixing. It will be seen that the higher the alkalinity of the medium of suspension, the greater is also the variation in the charge with concentration.

TABLE V

Effect of quinine bihydrochloride on 1 per cent suspension of R B C

Strength of the salt	$V \times 10^5$	
	In normal saline	In isotonic glucose solution
0	-16.2	-32.0
1 in 100,000	-15.9	-29.0
1 in 50,000	-15.5	-28.0
1 in 10,000	-14.5	-18.0
1 in 5,000		-8.0
1 in 1,000	-3.8 (within 10 mins) 4.8 (after $\frac{1}{2}$ hour) 3.5 („ 2 hours)	{ During hemolysis

TABLE VI

Effect of quinine bihydrochloride at different pH values on the charge of 1 per cent suspension of R B C in glucose solution

pH value of the buffer	$V \times 10^5$ AT DILUTION OF THE SALT				
	0	1 in 100,000	1 in 50,000	1 in 10,000	1 in 5,000
6	23.9	21.3	19.5	17.8	12.0
7	32.0	29.0	27.9	18.5	10.0
8	37.5	34.2	30.9	22.0	13.0

DISCUSSION

Physico-chemical—It is customary to interpret these data on cataphoretic velocity in terms of the Lamb-Helmholtz equation

$$\epsilon = \frac{4 \pi \eta v}{H D} \quad (3)$$

where $\frac{V}{H}$ gives the values given above (cataphoretic velocities), η the coefficient of viscosity, D the di-electric constant of the medium and ϵ the electrokinetic potential. When the values of D and η are taken to be constant, V is proportional to the potential ϵ at the interface. From our results we see that the cataphoretic velocity of erythrocytes suspended in normal saline is 16.2×10^{-5} cm per sec per volt per cm whereas the same in isotonic glucose solution is 32×10^{-5} in the same units. Now the di-electric constant of a solution of sodium chloride (0.85 per cent) is high and

that of an isotonic solution of glucose may be assumed to be low in the light of the experiments of Pechold(18), Furth(19) and Harrington(20) The former authors have shown that the D/C of a solution of an uni-univalent salt at first decreasing increases with the concentration of the salt while the latter has shown that non-electrolytes (one of which was cane-sugar) in general depress the di-electric constant of the water Writing equation (3) in a modified form and putting v' for v/H we see that

$$V'\eta = \frac{D}{4\pi} \quad (4)$$

from which we see that if the di-electric constant decreases, the velocity ought to decrease (viscosity differences between these solutions are negligible, so that they fail to explain the wide variation in the results) But we find that the cataphoretic velocity of erythrocytes in glucose solution is much higher though present in a medium of a lower di-electric constant Hence in order that the equation might hold good, we are to assume the rise of the potential by more than about 200 per cent, a possibility which on the very face of it appears to be doubtful

It appears, therefore, better to interpret these results in terms of the density of charge on the surface than in those of the potential Moreover equation (3) or (4) has for its fundamental basis a term involving the density of charge, from which with further assumptions (whose validity has been questioned by different investigators) they, in the forms given above, have been deduced In the light of modern researches in colloidal chemistry (21, 22, 23, 24 & 25) into which we need not go further, it has been becoming more and more clear that the cataphoretic velocity should preferably be interpreted in terms of the density of charge on the surface

It is, therefore, sufficient to know for our purpose that the cataphoretic velocity is proportional to the density of charge on the surface of erythrocytes The origin of the charge of such particles and their variation in presence of electrolytes and non-electrolytes can be best understood in the light of the theory of the double layer due to Mukherjee(26) A colloidal particle or a particle of suspension in its process of formation absorbs a certain species of ions on its surface because of its residual chemical affinity Due to the charge so acquired, a number of oppositely charged ions are attracted towards the surface, some of which remain bound and others are free depending on the concentration of oppositely charged ions The number of ions, which have a kinetic energy greater than that corresponding to the work W , are free, where

$$W = \frac{n_1 n_2 E^2}{Dx} \quad (5)$$

in which n_1 is the valency of the primarily absorbed ion, n_2 the valency of the oppositely charged ion, E the electronic charge, D the di-electric constant and x the distance between the centres of the ions at the point of their nearest approach

The density of charge will then be determined by the number of primarily absorbed ions per unit surface and the number of oppositely charged bound ions on the same area and their respective valencies. It is very difficult to suggest definitely the cause of the origin of the charge on the surface of erythrocytes, but it is possible that the primarily absorbed ions might be complex organic anions or hydroxyl ions or both. When suspended in normal saline, it is evident that depending on the concentration of sodium ions, a number of them will be bound and thus the charge acquired in such a solution will naturally be much less than that in isotonic sugar solution where practically the number of oppositely charged ions (i.e., sodium) is nil. The effect of the change of the hydrogen ion concentration will also be less marked in sodium chloride solution, where the di-electric constant is high (therefore absorption of bound ions will be low) and the number of places to be covered by hydrogen ions is less. Whereas in isotonic sugar solution where the di-electric constant is low and the number of bound ions already present are few, the hydrogen ions will at once be attracted and the charge, and hence the cataphoretic velocity, will decrease to a greater extent. With increase in hydroxyl ion concentration, the charge will increase more rapidly, since these hydroxyl ions will be chemically absorbed by the erythrocytes much more easily than in sodium chloride solution, where they have to pass through a number of bound sodium ions which will naturally hamper their progress towards the surface.

The effect of the addition of the quinine bihydrochloride can also be explained similarly. Comparing the results in presence of saline and isotonic sugar solutions, we see a higher absorption of the positive alkaloidal ion in presence of sugar which is what we expect because of the lower di-electric constant in the case of isotonic sugar solutions. The time effect on the charge, i.e., the variation of the charge and its subsequent reversal with time in 1 in 1,000 quinine bihydrochloride solution can also be explained from this point of view. It will be seen that at this concentration, partial hæmolysis takes place, which shows that the surface of the erythrocytes is undergoing some change and some time must elapse before a fresh equilibrium between the ions on the surface of the erythrocytes and those in the solution is reached. The concentration of positive alkaloidal ions is here high, so that they are able to reverse the charge of the erythrocytes. Next as with increase in alkalinity of the medium a higher density or negative charge is acquired by the erythrocytes, we find also a greater absorption of the alkaloidal ions. The number of positively charged alkaloidal ions attracted towards the surface will naturally be higher, where the initial density of charge is high. It is hoped that these few remarks will suffice for the elucidation of the facts mentioned above.

BIOCHEMICAL POINT OF VIEW

The fact that on the alkaline side a higher absorption of the alkaloidal ion takes place, supports the view put forward by the authors(13) that the higher toxicity

of the quinine alkaloids in alkaline solutions might at least in part be attributed to the higher negative charge acquired by *Paramœcrum caudatum* in alkaline solutions. From these and other considerations set forth in our previous paper (*loc cit*) we can also understand why there is higher concentration of quinine when administered with alkali. It is to be remembered that in concluding that paper, we noted 'it is difficult to explain the action of the drug itself on the malarial parasites, though a greater curative effect in alkaline solutions can be correlated with higher rate of absorption by the intestinal cells with the consequent greater concentration of quinine in the mesenteric blood. Morgenroth(27), however, put forward an interesting theory that quinine and the related alkaloids, when added to blood, accumulate in or on the red blood corpuscles, the serum or plasma retaining only a low proportion. Contrary to this Acton and King(28) find an equal distribution between serum and red blood corpuscles. Analytical determinations, however, give an imperfect idea of electrical absorption. It would be interesting, therefore, to see the effect of these low concentrations on the density of electrical charge of the red blood cells at different pH values'. These determinations have been carried out and, though they certainly explain partly at least the higher toxicity of these drugs towards *Paramœcrum caudatum* or the presence of higher concentration of the quinine in the blood when administered with alkali, it will be seen that those concentrations of the alkaloidal salt (1 in 100,000 and lower), which are obtained in the blood, have virtually little or no apparent effect on the negative charge of the erythrocytes when they are suspended either in normal saline or even in isotonic glucose solution. It is, therefore, expected that in the blood which is a much more complicated system and where in addition to sugar and sodium chloride, there are present other organic and inorganic substances, the negative charge of the red blood corpuscles should not change in presence of these low concentrations of alkaloid.

This fact, then, appears to go against the theory of the activity of quinine salts as suggested by Morgenroth (*loc cit*) and appears to confirm that of Acton and Chopra(29) who think that quinine salts have no direct action on the malarial parasites, at the concentrations of the salts that are generally obtained in the blood, their action being only to retard the growth and development of more malarial parasites. This conclusion is, however, to be accepted with caution as there are no data on the time effect of these low concentrations of salts on the cataphoretic velocity of erythrocytes from day to day.

SUMMARY AND CONCLUSIONS

1 The negative electrical charge of erythrocytes in isotonic glucose is more susceptible to changes in ionic environment than in normal saline solution. The higher the alkalinity of the environment, the greater is the negative charge.

2 The greater diminution in the negative charge on the alkaline side of the medium in presence of a definite concentration of quinine bihydrochloride shows a relatively higher absorption of basic alkaloidal ions

3 These facts suggest that the higher activity of quinine salts in an alkaline solution is at least partly due to the relatively higher absorption of the alkaloidal ions in such a medium

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STUDIES ON INDIAN SIMULIIDÆ

Part III.

DESCRIPTIONS OF MALES, FEMALES AND PUPÆ OF *S. GRISEIFRONS* BRUNETTI (1911) AND OF FOUR NEW SPECIES WITH STRIPED THORAX

BY

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[Received for publication, December 19, 1931]

THE five species dealt with in this paper are all characterized by having a striped thorax and possess simple claws in the female. They all belong to the subgenus *Simulium* and together with the species described already, in Parts I and II of this study, form a separate group, members of which differ from practically all other species of this subgenus, so far known from India, in having the anterior surface of the tibiæ conspicuously silvery white. The only exception appears to be *S. indicum*, which, however, can easily be distinguished from all these by the golden pubescence on its frons, which is practically bare in the species forming the above group.

***Simulium (Simulium) lineatum* SP. N**

FEMALE

Head black with short slender, dark hairs on the occiput, present though scanty on the face and a few also along the lateral borders of the frons. Frons somewhat shining black, nearly parallel sided, being only slightly narrowed in the region of the antennæ, its width about the middle is a little less than half its length. Face dusted with ash grey. Antennæ. In the type specimen the scape, three basal segments and the base of the 4th segment of the flagellum are orange yellow, while the rest is nearly black with very fine pale pubescence. The colour of the

basal portion of the antenna, however, varies in the different paratype specimens from yellow to red and reddish brown. In the majority of them the whole of the antenna is somewhat reddish, the basal one-third is dark red, gradually becoming reddish brown to nearly black towards the tip. Palpi nearly black.

Thorax—Mesonotum covered with golden pubescence, partly rubbed off on the dorsum in the type specimen. When viewed from in front the mesonotum* is grey with a narrow median and two broader submedian black stripes forming a lyre-shaped pattern. The median line does not reach the anterior border while the two sub-median stripes are continued along the anterior border and are connected to the still broader stripes running a little internal to the lateral border. The outermost stripes are about twice as wide as the sub-median ones which again are double the width of the median stripe. All the five black lines are connected to each other practically in level with the wing bases. The colours are as usual reversed when the mesonotum is viewed from behind. Scutellum is brownish black, covered with golden pubescence and long, black, marginal hairs. Pleuræ dark grey, membranous area bare.

Abdomen—The first segment brownish, the basal scale yellowish brown and the fringe of hairs golden, second segment yellowish brown with light grey dusting on dorsum, the rest dark brown. Tergites of segments 6-8 shining and nearly black, with short and slender pale hairs scattered on them, some present also on the anterior segments. Venter somewhat pale brown. *Terminalia* (Plate LV, fig 1). Hairs on the ventral surface of segment seven, uniformly distributed, sternite of segment eight with narrow lateral ends, posterior border somewhat rounded, fairly strong macrosetæ on the antero-lateral portions, the middle one-fourth bare. Anterior gonopophyses uniformly covered with microsetæ but, with on each, one to three macrosetæ only. Their interno-lateral borders are not thickened and are widely separated, considerably diverging posteriorly. Paraprocts and cerci are of moderate size, the former chitinized only on the outer side.

Legs—Fore coxæ and trochanters pale yellow, the latter greyish distally, femora yellow with the tip greyish, tibiae also yellow with the distal one-fifth black, the outer surface with a large silvery white spot, tarsi black, moderately flattened, the first segment about 5 times as long as its greatest width at the distal end. Segments one and three with the usual pair of long black hairs, subterminally on their posterior border. Middle and hind coxæ dark grey (nearly black),

* The pattern found on the mesonotum of this species differs from that in *christophersi* in that the outermost stripes lie not along the lateral border but a little internal to it, and also in that this stripe is about twice as wide as the sub-median one, unlike those of the latter species in which they are nearly equal in width. From that found in *griseifrons* it differs in that the median stripe is not exceptionally narrow, the sub-median stripes are more or less uniformly broad throughout their length, the outermost stripe runs a little internal to the lateral border and is connected to the sub-median line along the anterior border.

trochanters yellowish with dark distal ends, femora¹ greyish yellow with dark brown tips, tibiae pale yellow with the tip of that of the middle and the distal one-fourth of that of the hind leg black, posterior surface of both extensively silvery white. Basal half of the first tarsal segment of middle leg and a little more than the basal half of the first and the base of the second tarsal segment of the hind leg yellowish, the rest of the tarsi black. All yellow parts of the legs with fine pale golden pubescence. Pedisulcus well marked, calypala of moderate size, extending up to the former. All claws simple, without a sub basal tooth.

Wings—Normal, hyaline, radius bare up to the fork, radial sector simple, concave vein. Halteres pale yellow.

Wing-length nearly 3 mm

Posterior end of buccal cavity without any cluster of protuberances on the ventral surface. The posterior border is, however, thickened into a chitinous rim and produced into the pharyngeal cavity as a small truncated cone-like process with smooth lateral walls.

Furca of the usual form and spermatheca single, globular and dark brown.

MALE

Head black, with a fringe of short black hairs on the occiput. Face whitish grey, with sparse black hairs along its borders. Antennae reddish, becoming black gradually towards the tip, with very fine whitish pubescence. Palpi brownish black.

Thorax—Mesonotum is covered uniformly and densely with short golden pubescence, somewhat rubbed off in the middle in the type specimen. When viewed from in front the mesonotum appears dusted with greyish white with narrow black longitudinal lines showing the same pattern as in the female but the lines are comparatively much narrower. When viewed from behind the colours are reversed and the mesonotum appears velvety black with narrow grey lines. In discoloured and badly rubbed specimens this pattern becomes very faint and is not clearly seen, the portion at the anterior end being only visible. In the antero-lateral corners are a pair of large elongated silvery spots which are seen whole and not only half at a time. In certain lights the lateral as well as the posterior borders of the mesonotum appear conspicuously silvery. Scutellum is black, covered with golden pubescence and has a fringe of long black hairs. Pleurae with whitish grey sheen, membranous area bare.

Abdomen—Velvet black, with scattered very fine pale hairs. Silvery spots as usual on segments 2 and 5-7, those on segments 6 and 7 connected with each

* In specimens from Mercara (Coorg) the legs are comparatively darker. The middle and hind femora are extensively dark grey, latter being yellowish only near its base. Except in the colour of the legs these specimens resemble those from Marianbarie in every detail.

other forming a continuous curved spot, long hairs on the black basal scale dark brown. *Genital armature* (Plate LV, fig 2) —The coxites are short comparatively broader than they are long, styles of moderate length about three times as long as their greatest width near the base. Distal to their widest part in the basal one-third, they are somewhat narrowed widening again only slightly near their posterior end. From their dorsal surface near the inner edge, about one-third from the base, each sends upwards a hollow horn-like process which is about one-third the length of the styles. The anterior surface of these processes is raised into very small teeth-like protuberances. Each style bears a single short subterminal spine on its inner edge. The inter-coxal piece (Plate LV, fig 3) has a moderately broad base from which a flattened, narrow, somewhat tapering, tongue-like process is produced downwards and bent forwards. It is slightly expanded at its distal end. The ventral surface of the inter-coxal piece is bare and smooth, except the proximal broad portion on which there are always some transverse wrinkles and its lateral edges too are irregular, being broken up by some broad serrations. The spines forming a cluster on each side of the genital opening are comparatively very large.

Legs —Fore coxæ, trochanters and femora greyish yellow, the last dark grey near the distal end, tibiæ yellowish, distal half black, nearly the whole of the anterior surface silvery white, tarsi black moderately expanded, segment 1 about 6 times as long as its greatest width near the distal end. Middle coxæ black, trochanters yellowish grey, femora greyish yellow (darker than the fore femora) dark grey near the tip, tibiæ pale yellow with a black tip, the posterior surface with a silvery white sheen, basal half of the first tarsal segment yellow, the rest of the middle tarsi black. Hind coxæ black, trochanters yellowish grey, basal one-third of femora yellowish, gradually becoming black distally, tibiæ pale yellow on the basal one-third, the rest black, with a whitish sheen on the posterior surface of the basal portion, basitarsus pale yellow on the basal half, the rest of the tarsi black. The hind basitarsus (Plate LV, fig 4) is moderately enlarged. It is a little shorter than the tibiæ (0.8 of the length of the latter) and its proportionate width comparatively a little broader, its greatest breadth being 0.27 of its length.

* The amount of black pigment on the middle femora and hind femora and tibiæ is very variable in the various paratype specimens. The distal one fourth to about three fourths of the middle femora may be dark grey while the hind femora vary from being nearly all black to having the black pigment only near the tip. The distal half to practically the whole of the hind tibiæ may be black, when the latter, only the posterior surface of the extreme base is whitish.

In specimen with very dark middle and hind legs the greyish pattern on the mesonotum is not well developed. In the males of this species from Coorg, Mercara, and one specimen from Marianbarie even the fore femora are yellowish black and the middle femora and hind femora and tibiæ are nearly black. The mesothorax is velvet black without any grey lines having only two large anterior grey spots.

(while the greatest width of the tibiae is a trifle less than one-fourth its own length)
 The yellowish portions of the legs bear golden pubescence
Wings as in female

PUPA

Size about 3.1×1.0 mm

The integument of the head and thorax is brown closely covered with comparatively very minute tubercles, they do not appear disc-like. The head bears the usual three pairs of trichomes which are moderately long and simple. Unlike the species dealt with already (in parts I and II of this study) the thorax bears 5 instead of 4 pairs of trichomes dorsally. These are fairly long and may be simple or split a little above their base into two or three branches. The cuticular hooks on the dorsal as well as on the ventral surface are as in *himalayense*, but without any strongly chitinized sensory hair or hook on ventral surface of segment 4. Dorsally on segment 8 only, there is a continuous row of backwardly directed short cuticular spines. The subterminal spine on segment 9 is absent.

Respiratory filaments (Plate LV, fig 5) are a little less than half the length of the pupa, their length being about 1.4 mm, 8 in number, arranged in 4 pairs all of which have short stalks. The uppermost filament is directed upwards and a little forwards from its origin while the lowermost passes downwards the rest of the filaments spreading out more or less evenly between these two. The filaments decrease in thickness slightly from above downwards. The surface of the filaments is raised into ridges which form a reticular pattern, the ridges covered with large tubercles while the interspaces with minute ones resembling the arrangement found in *malguncum*.

Cocoon (Plate LV, fig 6) about 3.6×1.5 mm, not covering the pupa completely, as its length dorsally is only about 2.6 mm. It is dirty, brownish yellow, closely woven, with a large elongated window on each side. These windows run parallel to the anterior border, lying just behind it on each side. Usually they are of the same shape though in some specimens they are comparatively smaller. In a large number of specimens each of the windows is divided into two by a narrow horizontal bar. The anterior border of the cocoon is thickened into a moderately strong rim.

Described from 24 females and 20 males all bred out of isolated pupae and in good condition.

Types in my own collection

DISTRIBUTION

So far I have bred out specimens of this species from isolated pupae collected from hill streams near Marianbarie, Bengal Terai, 1 in 29 (types) and from a

* Very often present on the pupae of *himalayense*

stream crossing the Mercara-Cannanore Road about three miles below Mercara (Coorg), 9 1 31 In the Indian Museum collection there are three male specimens in good condition, collected by 'C Paiva,' from 'Almora, Kumaon (5,500 feet), 28 ix 11'

Simulium (Simulium) barraudi SP N

This species closely resembles *S lineatum* from which the female is difficult to differentiate The male genitalia are, however, so distinct that there can be no doubt about the separate identity of this species

FEMALE

The female differs slightly from that of *S lineatum* only in the colour of its legs, the head, thorax and abdomen (the terminalia as well) being as in the latter species

Legs—Fore coxæ, trochanters and femora yellow, the last named slightly greyish at its distal end, tibiæ yellow, the outer surface with a large silvery white spot and the distal end (about one-sixth) nearly black, tarsi black, much flattened, the first segment only four times as long as its greatest width near the distal end Middle and hind coxæ black, trochanters orange yellow* Femora yellow with a slight greyness at the distal end Middle tibiæ pale yellow with the posterior surface silvery white and the distal end slightly grey, basal three-fourths of the first tarsal segment pale yellow with a slight whitish sheen on the posterior surface, the rest of the middle tarsi black Hind tibiæ yellow with the posterior surface silvery white and distal one-fifth black, basal three-fourth of the first tarsal segment yellow, having a slight whitish sheen on the posterior surface and the distal one-fourth gradually becoming black, base of the second segment yellowish, the rest of the tarsi black All claws simple All yellow parts of the legs with fine golden pubescence

Wings normal, radius bare up to the fork, radial sector simple Wing length in the type specimen is about 2.5 mm (average wing length in the paratype specimens is 2.7 mm)

Posterior end of buccal cavity as in *lineatum*

MALE

Head black, with a fringe of black hairs on the occiput, face whitish grey, with sparse black hairs along its borders Antennæ yellow in the basal half, gradually becoming dark grey towards the tip with a fine pale pubescence The colour of the antenna varies in the various paratype specimens from orange or reddish in the basal half, becoming black gradually towards the tip, to being nearly all black with only one to three basal segments slightly reddish Palpi black

* In paratype and other specimens the trochanters are yellow

Thorax—Mesonotum velvet black, covered uniformly and densely with coarse golden pubescence, partly rubbed off on the top in type specimen. Anteriorly are a pair of elongated pearly white spots broadly separated in the middle line. In certain lights even the space in between the spots appears ash grey but the stripes found on the female and on the majority of males of *lineatum* are absent in this species. Scutellum black covered with golden pubescence and having a fringe of long black hairs. Pleuræ slate grey, membranous area bare.

Abdomen—Velvet black, with scattered very fine pale hairs. Silvery spots as usual on segments 2 and 5-7, those on segments 5-7 elongated, long hairs on the basal scale black. *Genital armature* (Plate LV, fig 7) resembles that of *S. lineatum*. The coxites are short, broader than they are long, styles comparatively broader and a trifle longer than in *lineatum*. They are of more or less uniform width throughout, except at about two-thirds of their length from the base, where their outer border is produced upwards as a conspicuous broadly rounded projection. A hollow horn-like process arises dorsally from the basal one-third of the styles, as in *lineatum*, differing from that in the latter species only in that its distal end is slightly truncated and the protuberances on its anterior surface are small and rounded. The intercoxal piece (Plate LV, fig 8) is of the same general form as in *lineatum* but has comparatively broader base from which a broad, flattened tongue-like process is produced downwards and forwards. This process is comparatively broader and shorter and has a more or less uniformly rounded border. The antero-dorsal surface of the process bears a few scattered fine setæ. The chitinous plate of the mesosome has a somewhat different shape to that of *lineatum*, as it has a pair of small lateral projections at its posterior end.

Legs—Fore coxæ pale yellow, trochanters and femora yellow, the latter slightly greyish distally, tibiæ blackish, with a large silvery white spot on its anterior surface and outer border, tarsi black, moderately flattened, the first segment a little less than six times as long as its greatest width near the distal end. Middle and hind coxæ black, trochanters yellowish grey, dark in the distal half. Middle femora and tibiæ yellow, slightly greyish distally, the latter with a whitish sheen on their posterior surface, basal one-third of the first tarsal segment yellowish, the rest of the middle tarsi black. Hind femora yellow near the base, distally becoming gradually brown to nearly black at the tip, tibiæ nearly black with a whitish sheen on the posterior surface of the basal portion, basal half of the basitarsus yellowish, the rest of the tarsi black. The hind basitarsus is moderately enlarged. It is a little shorter than the tibiæ and proportionately of the same width (0.8 of that of the latter), its greatest width is about 0.3 of its own length.

Wings as in female.

J, MR

PUPA as in *S. lineatum*

Described from 12 males, 9 females, and a large number of pupæ, practically all in good condition, the adults bred out of isolated pupæ

Types and paratypes in my own collection

DISTRIBUTION

Specimens of this species were bred out of isolated pupæ collected by Colonel Sir S R Christophers and Captain P J Barraud, Entomologist (*Malaria Survey of India, Kasauli*), from a large stream near Prang (6,500 feet), and a torrential stream at Nara Nag (about 7,500 feet above sea-level) both in Kashmere. I have so far bred specimens of this species out of pupæ collected from the following places. From small streams crossing the Kasauli-Subathu Road (4,000-5,000 feet), 15 viii 26, a hill stream north-east of Dagshai (about 5,000 feet), August 1929, from the stream north of Chhota Simla (about 6,000 feet), 6 ix 30, from Chadwick Falls (about 5,500 feet), 7 ix 30, from streams crossing the Hindustan-Tibet Road near Theog and Matiana, ranging in height from 6,800-8,200 feet, 11-13 ix 30. The pupæ were found breeding together with those of some other species, in some places as many as 8 species occurring in the same locality.

I am pleased to name this species after Captain P J Barraud, Entomologist (*Malaria Survey of India, Kasauli*) who together with Colonel Sir S R Christophers collected simuliids for me from parts of Kashmere.

Simulium (Simulium) digitatum SP. N

This species closely resembles *S. lineatum* and *barraud* from both of which it differs mainly in the colour of its legs and in the terminaha of the male and the female.

FEMALE

Head black, with short, black hairs on the occiput, some present also on the face and a few along the lateral borders of the frons. Frons somewhat shining, nearly black, practically parallel sided, only very slightly narrowed in the region of the antennæ, its width about the middle a little more than half (0.6 of) its length. Face dusted with ash grey. Antennæ reddish brown in the basal portion, gradually becoming darker towards its distal end which is nearly black, the whole with a fine pale pubescence. The colour of the antennæ, particularly of the basal segment, varies in the different paratype specimens. The scape and the bases of two or three flagellar segments may be yellow to orange and the rest of the antennæ nearly black. Palpi nearly black.

Thorax—The ornamentation of the mesonotum is like that in *lineatum* and *barraud* except that the median line is comparatively much narrower.

Abdomen —The first segment dark brown and the fringe of long hairs on the basal scale golden (mixed with some black ones), the rest of the abdomen nearly black, the second segment with light grey dusting on the dorsum. Tergites of segments 6-8 shining black, with scattered fine pale hairs. Venter black (as in *barraudi*). *Terminalia* (Plate LV, fig 9). Hairs on ventral surface of segment 7 uniformly distributed, sternite of segment 8 comparatively narrower than in *lineatum* and *barraudi*, with narrow lateral ends, posterior border broadly rounded, middle third nearly straight, macrosetæ comparatively slender, only a few in the lateral region very long, the rest gradually decreasing in size proceeding towards the middle line. A few macrosetæ present even on the middle fourth of the sternite. Anterior gonopophyses uniformly covered with microsetæ and with 7-9 slender macrosetæ scattered on them. Their interno-lateral borders are not thickened and are widely separated from each other. Paraprocts and cerci are of moderate size.

Legs —Fore coxæ and trochanters yellow, femora yellow, gradually passing into dark grey on the distal one-third, tibiae yellow with the distal one-fifth black, the outer surface with a large silvery white spot, tarsi black, much flattened, the first segment about four times its greatest width near the distal end. Segments 1 and 3 with the usual pair of long black hairs subterminally on their posterior border. Middle and hind coxæ nearly black, trochanters yellowish grey, dark on the distal half. Middle femora greyish yellow gradually passing into grey on the distal half. In the paratype specimens the legs are darker and the middle femora are yellowish grey on the basal one-third passing into dark grey distally. Middle tibiae are pale yellow, with the distal end dark grey, the posterior surface with a whitish sheen, basal three-fourths of the first tarsal segment pale yellow, the posterior surface with a slight whitish sheen in the basal region, the rest of the middle tarsi black. Hind femora yellowish on the basal one-third, passing into dark brown distally, tibiae yellow with distal one-fourth black, the posterior surface with a whitish sheen, basal three-fourths of the first and the base of the second tarsal segment yellow, the rest of the tarsi black.

Wing —Normal, hyaline, radial sector simple.

Wing length about 2.5 mm.

Projection of the posterior border of the ventral wall of buccal cavity into pharynx as in *S. lineatum*.

MALE

Head and thorax as in *barraudi*. The antennæ in the type are black with the scape and the first flagellar segment yellowish.

Abdomen —Velvet black, with scattered very fine pale hairs, silvery white spots as usual on segments 2 and 5-7, those on segments 5-7 elongated, long hairs on basal scale black. *Genital armature* resembles that of *S. lineatum*, differing

from the latter in certain details and in the form of the inter-coxal piece. The coxites are about as long as they are broad. The horn-like process arising from the dorsal surface of the basal one-third of the styles (Plate LV, fig. 10) is a little more than one-third the length of the latter. Their anterior straight surface is more or less smooth practically free from any teeth and the distal end of the process is pointed and slightly bent. The inter-coxal piece (Plate LVI, fig. 11) has a moderately broad, somewhat swollen base from which a narrow finger-like process with a rounded end is produced downwards and only slightly forwards. It is smooth except for a few wrinkles on its ventro-posterior surface.

Legs—Fore coxæ and trochanters yellow, femora yellowish grey with the distal end dark grey, tibiæ black, with a large silvery white spot on the outer surface, tarsi black moderately flattened, first segment a little more than 5 times its greatest width near the distal end. Middle and hind coxæ black, trochanters yellowish grey. Middle femora brown, yellowish near the base, tibiæ yellow gradually becoming brown on distal half, posterior surface with a whitish sheen, basal half of the first tarsal segment yellow gradually passing into black distally, the rest of the middle tarsi black. Hind femora nearly black with the basal one-third yellowish, tibiæ black with a slight whitish sheen on the posterior surface near the base, basal half of basitarsus and base of second tarsal segment yellow, the rest of the hind tarsi black. Basitarsus moderately enlarged a little shorter and narrower than the tibiæ, comparatively broader than in *lineatum*, its greatest width being about 0.32 of its own length. The yellowish portions of the legs bear golden pubescence. The femora differ from those of *lineatum* and *barraudi* in having a larger number of black hairs.

Wings as in female.

PUPA

The pupa resembles those of *S. lineatum* and *barraudi* except that a sensory hair on the ventral surface of abdominal segment 4 is somewhat strongly chitinized. Moreover, the colour of the respiratory filaments, instead of being greyish as in the other two species, is usually white.

Described from 3 males and 5 females, all bred out of isolated pupæ and practically all in good condition.

Types and paratypes in my own collection.

DISTRIBUTION

I have bred out this species from pupæ so far collected from a small stream crossing the Kasauli-Subathu Road (about 4,500 feet above sea-level), 15 viii 26, a hill stream north-east of Dagshai (about 5,000 feet), August 1929, a stream north of Chhota Simla (about 6,000 feet), 6 ix 30 and from Chadwick Falls (about 5,500 feet), 7 ix 30.

Simulium (Simulium) dentatum SP. N

This species closely resembles the three described above, differing from them in being comparatively much darker and in certain details in the structure of the terminalia of both the sexes

FEMALE

Head and *thorax* are like those of *lineatum*. The antennæ, however, are nearly black with the scape and two basal flagellar segments slightly brownish, the whole with very fine pale pubescence. Colours of the basal segments vary in the paratype specimens from greyish yellow to somewhat reddish brown.

Abdomen is also like that of *lineatum* but with a dark venter and somewhat different terminalia. The 8th sternite (Plate LVI, fig. 12) is of the same type and bears similar macrosetæ but the bare portion is comparatively smaller and two or three short setæ are present even on the middle fourth near the anterior border of the sternite. The anterior gonopophyses are uniformly covered with microsetæ and bear 5-9 moderately long and slender macrosetæ. They are widely separated and their interno-lateral border is not thickened.

Legs—Fore coxæ pale yellow, trochanters and femora deep yellow, the latter dark brown on the distal half, tibiæ yellow, with distal one-fourth black and a large silvery white spot on the outer surface, tarsi black, much flattened, the first segment a little less than four times its greatest width near the distal end. Middle and hind coxæ nearly black, trochanters yellow, slightly greyish distally. Middle femora brownish yellow on the basal one-third, gradually becoming dark brown distally, with the tip nearly black, tibiæ pale yellow with the distal one-fifth dark brown and with a slight whitish sheen on the posterior surface basally, basal half of the first segment pale yellow, the rest of the middle tarsi black. Hind femora dark brown (nearly black) with the base pale yellow, tibiæ pale yellow with the distal one-fourth nearly black and with a whitish sheen on the posterior surface basally, basal two-thirds of the first and the base of the second tarsal segment yellow, the rest black. Pale portions of the legs with golden pubescence. Pedisulcus and calcipala well marked. All claws simple.

Wings hyaline, radius bare up to the fork, radial sector simple, concave vein.

Wing length about 2.7 mm.

The posterior border of the buccal cavity thickened and produced as a truncated cone-like projection into the pharyngeal cavity, as in *lineatum*, but in one of the paratype specimens the sides of the projection seem to have minute setæ on them.

MALE

Head black with a fringe of short black hairs on the occiput. Face whitish grey with sparse black hairs. Antennæ yellowish or reddish-becoming black.

gradually towards the tip In some paratype specimens they are black practically throughout their length Palpi brownish black

Thorax —Mesonotum velvet black with a slight brownish or reddish sheen, the satin rubbed off in the type specimen from part of mesonotum, giving that portion a shiny appearance Mesonotum covered uniformly and densely with a coarse golden pubescence, partly denuded in the type In the fore corners are a pair of long, elongated whitish grey spots which are seen complete and in certain lights fairly wide whitish grey band is seen along the lateral and posterior border also Scutellum is reddish brown with coarse golden pubescence and a fringe of long black hairs Pleuræ slate grey, membranous area bare

Abdomen velvet black with fine scattered golden pubescence, long hairs on the basal scale black, segments 2 and 5-7 with the usual silver grey spots *Genital armature* The coxites are short about as long as they are broad, styles are comparatively longer than in *S. lineatum* and have a somewhat different shape to that found in the latter species They are a little less than four times as long as their greatest breadth near the base, and are slightly expanded in their basal one-fourth posterior to which they are gradually narrowed widening out again distally The hollow horn-like process given off from the dorsal surface has a comparatively wider base and lies much nearer the base of the styles (Plate LVI, fig 13) This process has a truncated end which is toothed and its anterior surface also bears conspicuous teeth which are much better developed than in *lineatum* or *barraudi* The inter-coxal piece (Plate LVI, fig 14) has a rather narrow base from which a flattened keel-like process, with two lateral rows of deep teeth-like serrations, is produced downwards It is continued forwards as a thinly chitinized somewhat expanded plate with smooth edges

Legs —Fore coxæ pale yellow, trochanters and femora brownish yellow, the latter becoming dark brown on the distal two-thirds, tibiæ brownish black, the outer surface with a large silvery white spot, tarsi black, moderately expanded, the first segment a little less than five times as long as its greatest width near the distal end Middle and hind coxæ black, trochanters yellowish black, femora nearly black, slightly yellowish at the base Middle tibiæ greyish yellow on the basal one-third, gradually becoming dark grey to nearly black distally and the posterior surface of the basal half with a whitish sheen, the basal one-third of the first segment of middle tarsi diffusedly yellowish, the rest of tarsi black Hind tibiæ black, with the base slightly pale yellow, basal half of the first and the base of the second segment of the hind tarsi greyish yellow, the rest of tarsi black Basitarsus of hind leg comparatively much enlarged, its length being about 0.8 of that of the hind tibiæ while its greatest width, a little beyond its middle, is a little more than that of the latter and 0.37 of its own length

Wings as in female.

PUPA

The pupa resembles that of *lineatum* except that the minute tubercles on the head and thoracic integument are comparatively much smaller and widely separated. The respiratory filaments too are whitish instead of grey.

Described from 10 males and 8 females all bred out of isolated pupæ and in good condition.

Types and paratypes in my own collection.

DISTRIBUTION

I have so far bred this species out of pupæ collected from hill streams near Marianbarie, Bengal Terai, 1 iii 28, streams in Kuseong (6,000 feet), Darjeeling District, August 1928. In the Indian Museum collection, there are 4 female specimens in good condition, belonging to this species collected by 'S Kemp', from 'above Tura, Garro Hills Assam, 3,500-3,900 feet, 15 vii -30 viii 1917'.

***Simulium (Simulium) griseifrons*, BRUNETTI (1911)**

= *Simulium diagrammicum* Edwards (1928)

This species was described by Brunetti (1911) from a single female collected by Dr A D Imms at Kalighat, Kumaon (6,000 feet), Western Himalayas, 4 iv 10. In the type specimen the thorax is badly discoloured and owing to some extraneous substance settled on it, it appeared slightly shining, and from the description given by Brunetti it is obvious that the specimen was in this condition even at the time (1911) when it was originally described*. By a very careful treatment of the thorax of the type specimen with a fine brush slightly moistened with absolute alcohol, I have been able to remove the substance which was settled on it and the thorax now shows its ornamentation very clearly. I have in my collection 4 male specimens and 6 females all bred out from pupæ collected from around Simla and from parts of Kashmere (Western Himalayas), all of which undoubtedly belong to this species. They resemble the type specimen in every character except in the colour of the antenna which does not seem to be constant. This identification is corroborated by a comparison of the terminalia of the female with those of the type specimen. In view of the inadequate and incomplete description given by Brunetti, it has been thought advisable to give below a revised description of the female.

FEMALE

Head slate grey, with short slender black hairs on the occiput, also present though scanty on the face and a few on the frons forming a row along its lateral

* Brunetti described the thorax as 'black, apparently covered with short golden yellow pubescence'.

borders Frons dull ash grey comparatively small, nearly parallel sided, only very slightly narrowed in the region of the antennæ Face dusted with grey

Antennæ —In the type female they are wholly black but in the other specimens, all bred out from pupæ, the colour of the antennæ from the scape to the base of the third flagellar segment, varies from deep yellow to reddish brown, the rest is black with fine whitish pubescence Palpi black

Thorax —Mesonotum covered with fine golden pubescence, integument dull When viewed from in front it appears dusted with ash grey with two rather broad black submedian stripes, extending from the anterior margin to the prescutellar region where they unite with each other practically in line with the bases of the wings A little behind their anterior end, which is fairly broad, these stripes are somewhat narrowed and approximated to each other, gradually broadening out again and diverging slightly behind their narrowest portion Between these two stripes is a very narrow median black line which commences from the union of the two posteriorly but does not reach the anterior margin A moderately broad black band extends along the lateral margins of the mesonotum uniting with the two submedian black stripes posteriorly but not at their anterior end As usual, when viewed from behind the grey and black colours are reversed Scutellum is black, densely covered with fine golden pubescence and has a fringe of long black hairs Pleuræ slate grey, brownish posteriorly, membranous area bare

Abdomen with first two segments slightly brownish, the fringe of hairs on the basal scale dark, dorsum of segment 2 with the usual greyish dusting, the segments following blackish, tergites of segment 6-8 large, black and shining, with scanty short hairs *Terminalia* (Plate LVI, fig 15) Macrosetæ on the ventral surface of segment 7 are uniformly scattered all over, sternite of segment 8 is fairly broad with its lateral ends slightly narrowed and bears a number of somewhat slender macrosetæ antero-laterally Anterior gonopophyses are of moderate size, their interno-posterior end somewhat rounded and inner border slightly curved and thickened Paraprocts are comparatively large and strongly chitinated and the cerci have a very broad base

Legs —Fore coxæ yellow, trochanters yellow to brownish yellow, femora yellow on the basal half, gradually becoming brown to dark brown apically*, tibiae yellow with the apical one-fourth black and well marked silvery dusting on the outer side, tarsi black, moderately flattened, first segment is about six times as long as its greatest width near the distal end Middle and hind coxæ black, trochanters yellow, slightly brown apically, femora yellow at the base, distal half to two-thirds black, tibiae pale yellow on the basal three-fourths, with a whitish sheen on its posterior surface, tip black, basal one-third to half of the first tarsal segment of middle leg and basal half of the first and base of the second

* In some specimens the tip is black.

tarsal segments of the hind legs yellowish, the rest of the tarsi black All claws simple Yellow parts of the legs with fine golden pubescence

Wings normal, hyaline, radial sector simple, concave vein The radius may or may not be hairy throughout its length In the type female the radius is bare up to the fork (and not hairy throughout as erroneously described by Edwards, 1928), but out of the six female specimens in my collection, bred out of isolated pupæ collected from parts of the Western Himalayas, in five of them the radius is hairy throughout while in one it is bare up to the fork These specimens resemble one another in all the other characters and they all undoubtedly belong to the same species Halteres pale yellow

Average wing length is about 3.2 mm (that in the type female is 3.38 mm)

The ventral wall of the buccal cavity at its posterior end is produced into the pharynx as a triangular piece which is covered with spinous processes Furca moderately expanded and of usual form, spermatheca single, dark brown and globular

MALE

Head black with a fringe of short black hairs on the occiput Face whitish grey with scattered black hairs Antennæ black with very fine whitish pubescence Palpi black

Thorax—Mesonotum velvet black, densely covered with rather coarse golden pubescence (partly rubbed off in the type specimen) Anteriorly are a pair of elongated silvery spots broadly separated in the middle and with a slight bluish sheen They are seen more or less complete In certain lights the mesonotum shows broad silvery border laterally, and a slight pale grey colour anteriorly between the two elongated spots, the latter only in the co-type specimen from Nara Nag Scutellum is black, densely covered with rather coarse golden pubescence and has a fringe of long black hairs Pleuræ slate grey, membranous area bare

Abdomen velvet black, with scattered golden pubescence and the usual silvery spots on segments 2 and 5-7 Long hairs on the basal scale somewhat golden *Genital armature* (Plate LVI, fig 16) Coxites appear somewhat broader than their length Styles are comparatively very long, their length being about 4 times their greatest width at the base Beyond the basal one-third they become narrow and broaden out slightly again at their distal end On the dorso-internal surface of their basal third each of them bears a triangular protuberance directed inwards and forwards (towards the base of the styles) These processes are covered with small teeth-like denticles Each of the styles bears a single short spine subterminally on the inner edge The inter-coxal piece (Plate LVI, fig 17) has a rather narrow base from which a very short narrow conical process projects downwards This process is bare and smooth

Legs —Fore coxæ brownish yellow, posterior ones black, trochanters yellowish at the base, dark grey on the apical half, femora brownish black, yellowish on the basal one-third and black at the apex. Fore tibiae are black, slightly yellow at the base and a large silvery white spot on their outer surface, tarsi black, moderately expanded, the first segment a little more than five times its greatest width at its distal end. Middle tibiae pale yellow on the basal half, with a whitish sheen on the posterior surface, distal half black, tarsi black. Hind femora black, diffusedly yellow basally and with a whitish sheen on the posterior surface at the base. Hind tarsi black, except the basal one-third of the basitarsus which is diffusedly yellowish. Basitarsus is much enlarged. It is only a trifle shorter than the tibiae, as broad as the latter and about half as broad as its own length. The legs bear scattered golden pubescence, well marked only on the pale parts.

Wings as in female. In all the four specimens the radius is hairy throughout its length.

Male described from two co-type specimens one hatched out of pupa collected at Nara Nag, 6,000 feet (Kashmere, Western Himalayas), by Colonel Sir S. R. Christophers, September 1930, and the other partly emerged from pupa collected by me from a torrential stream crossing the Hindustan-Tibet Road (Mile 29 from Simla), 8,000 feet, September 1930. Two other male specimens were dissected out of dead pupæ both collected at Nara Nag along with one of the co-type specimens.

Co-types in my own collection.

PUPA (Plate LVI, fig. 20)

Size about 3.0 mm × 1 mm

The integument of the head and thorax is brown with disc-like tubercles scattered all over, those on the anterior three-fourths of the mesonotum are comparatively very large (Plate LVI, fig. 18).

The head bears the usual three pairs of sensory hairs, and there are eleven pairs on the thorax. Like most of the European species the dorsal submedian group of trichomes on the thorax has three instead of two pairs found in the species described in Parts I and II of this study. The head and the dorsal trichomes (Plate LVI, fig. 18) divide a number of times near their base into long branches which vary in number from 18 to 30 and which spread out like a fan. The cuticular hooks on the dorsal as well as the ventral surface are as in *S. himalayense*, a single sensory hair is somewhat strongly chitinized on the ventral surface of segment 4. Dorsally on segment 8 there is a row, broken in the middle line, of backwardly directed cuticular spines along the anterior border, a few very small spines being also present on segment 7. The subterminal spines on segment 9 are comparatively better developed.

Respiratory filaments (Plate LVI, fig. 19) are about one-third as long as the pupa, 6 in number, arranged in three pairs, the uppermost of which has a very

short stalk while the other two arise more or less directly from the main stem. The uppermost filament is directed upwards and a little forwards and then bends downwards practically at right angle. The upper five filaments run more or less parallel to one another while the lowermost one is directed downwards from its origin. The filaments appear slightly whitish, their surface bears minute tubercles which are arranged as in *S. nigricum*.

Cocoon (Plate LVI, fig 20) is pale dirty yellow in colour. It is fairly tough and usually without any windows or spaces but some specimens show a small opening in the mesh laterally just behind the anterior border. The cocoon is boot-shaped, its opening is large and directed upwards, the forward extension of the ventral lip of the opening is comparatively thinner and is much enlarged. The length of the cocoon from its posterior end to the anterior border of the opening is about 4.2 mm and that up to the posterior border (of opening) only about 2.7 mm, the greatest width is about 1.3 mm.

DISTRIBUTION

This species was originally described from Kalighat, Kumaon (6,000 feet), Western Himalayas, and I have bred out a few specimens belonging to it from a number of isolated pupæ collected from Chadwick Falls, Simla, about 6,500 feet, 7 ix 30, torrential streams crossing the Hindustan-Tibet Road near Fagu, 14 ix 30 and, Matiana 11 ix 30 (heights ranging from 7,000 feet to 8,200 feet above sea-level). Colonel Sir S. R. Christophers collected a number of pupæ belonging to this species from a torrential stream near Nara Nag (6,500 feet), Kashmere, Western Himalayas, and bred a few isolated ones into adults.

Dr F. W. Edwards (Natural History Museum, London) has very kindly sent me a paratype female of his species *digrammicum* (Edwards, 1928). This specimen agrees with *S. griseifrons* Brunetti practically in every character. A very close comparison of its *terminalia* with those of the female of the latter species shows only very slight differences. In *digrammicum* the anterior gonopophyses and the paraprocts appear a trifle smaller and the latter a little less strongly chitinized and the cerci too a little less broad at the base. The differences, however, are not well marked and I am of opinion that *S. digrammicum* should provisionally be placed as a *synonym* of *S. griseifrons* till its male and pupa are definitely known to differ from those of the latter species.

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EXPLANATION OF PLATE LV

Simulium lineatum sp. n.

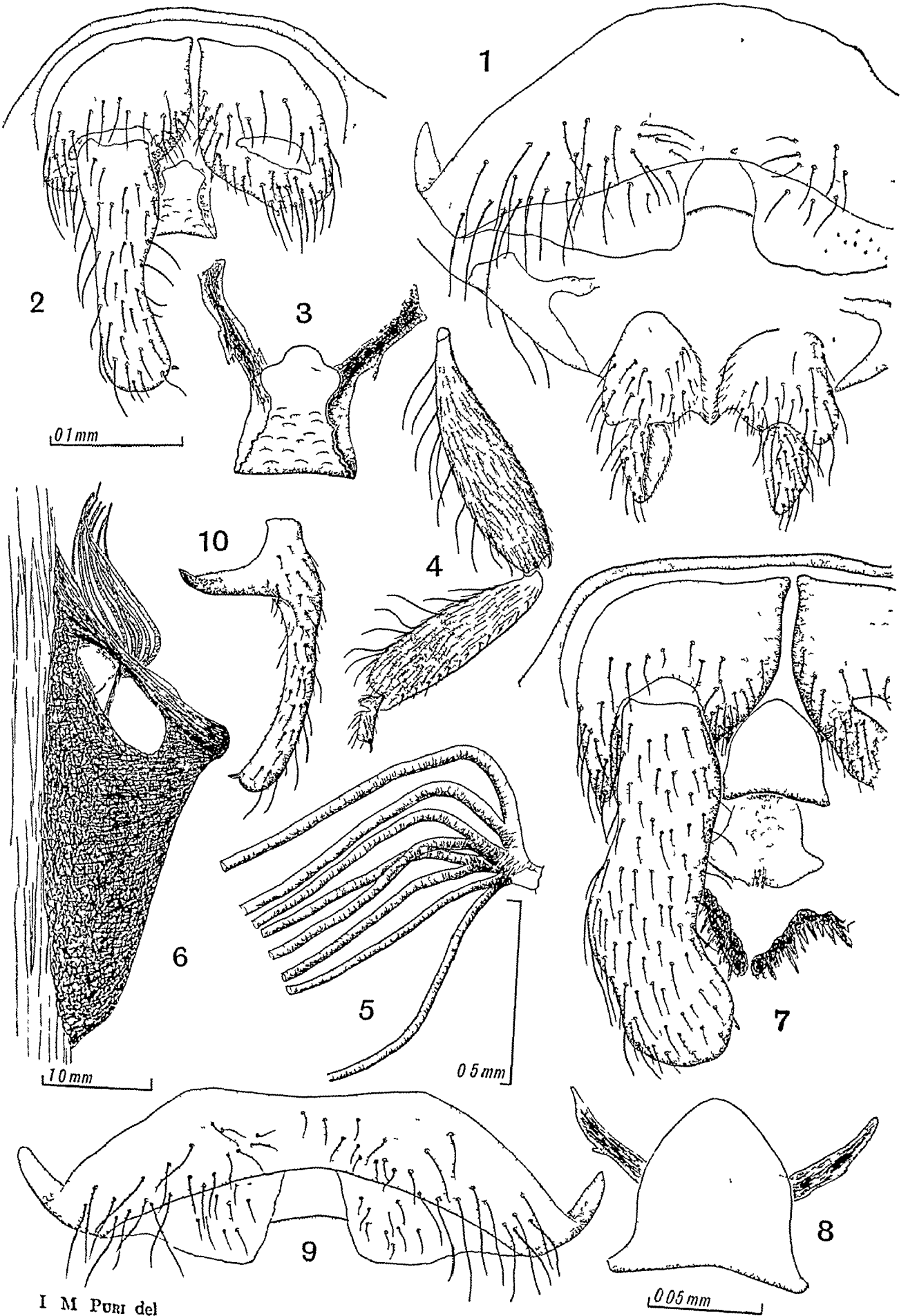
- Fig 1 Part of ventral view of terminalia of a paratype female Scale as in Fig 2
 „ 2 Ventral view of genital armature of a paratype male Left style and mesosome not shown
 „ 3 Ventral view of inter-coxal piece Scale as in Fig 8
 „ 4 Tibia, basitarsus and 2nd tarsal segment of hind leg of a paratype male Scale as in Fig 5
 „ 5 Parts of pupal respiratory filament of left side
 „ 6 Lateral view of pupa inside cocoon

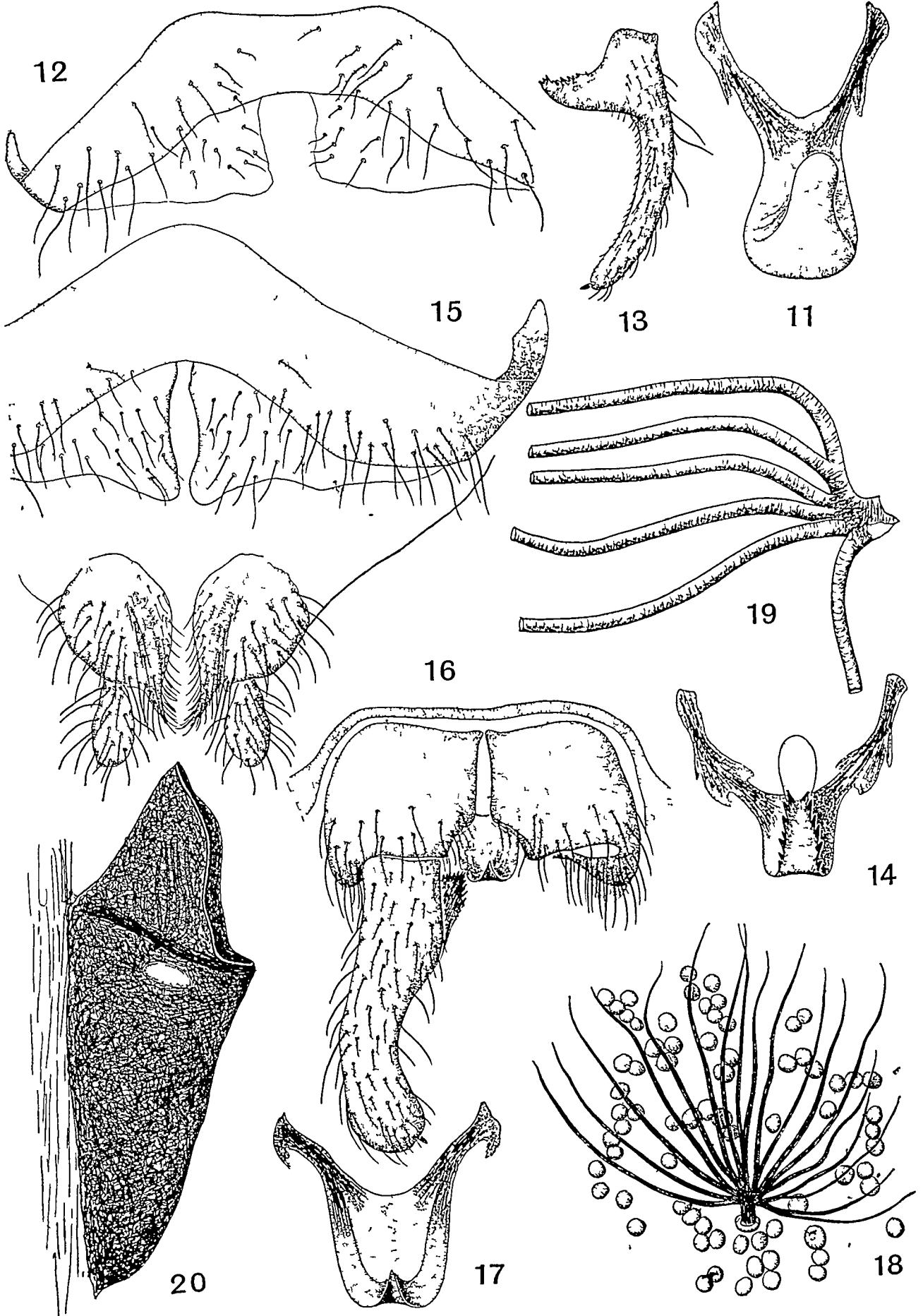
Simulium barraudi sp. n.

- „ 7 Ventral view of genital armature of co-type male from Nara Nag Left style not shown Scale as in Fig 2
 „ 8 Ventral view of inter-coxal piece

Simulium digitatum sp. n.

- „ 9 Sternite of segment 8 and anterior gonopophyses of a paratype female Scale as in Fig 2
 „ 10 Lateral view (inner) of a left style of a paratype male Scale as in Fig 2.





EXPLANATION OF PLATE LVI

Simulium digitatum sp. n.

Fig. 11 Ventral view of inter-coxal piece of a paratype male Scale as in Fig. 8

Simulium dentatum sp. n.

„ 12 Part of sternite of 8th segment and anterior gonopophyses of a paratype female Scale as in Fig. 2

„ 13 Lateral view (inner) of left style of a paratype male Scale as in Fig. 2

„ 14 Ventral view of inter-coxal piece of a paratype male Scale as in Fig. 8

Simulium greseifrons Brunetti (1911)

„ 15 Ventral view of part of terminalia of a female Scale as in Fig. 2

„ 16 Ventral view of part of genital armature of co-type male Scale as in Fig. 2

„ 17 Ventral view of inter-coxal piece of paratype male Scale as in Fig. 8

„ 18 A thoracic trichome of pupa Scale as in Fig. 2

„ 19 Parts of pupal respiratory filaments Scale as in Fig. 5

„ 20 Lateral view of pupa inside cocoon Scale as in Fig. 6

FURTHER OBSERVATIONS ON SEASONAL VARIATION IN HOOKWORM INFECTION.

BY

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[Received for publication, December 28, 1931]

IN a previous paper (Maplestone, 1930) it was shown that in two tea garden areas, one in the Bengal Dooars and the other in Assam in the Sylhet district, there was considerable annual variation in the number of hookworm eggs passed by a given sample of the population, and that the differences noted seemed to be clearly correlated with the onset and increase of the monsoon. Similar observations have now been completed over a period of twelve months at a large jute mill and its surrounding villages, situated near the Hooghly river about twenty miles from Calcutta.

For purposes of comparison, the area examined was divided into three sections as follows. Section 1 consisted of the lines directly under control of the jute mill and situated on their property, section 2 was a rather insanitary and crowded village adjoining the jute mill boundary, and section 3 was another village, this appeared to be somewhat less crowded than section 2 and was also cleaner. In section 1 there is an efficient pan conservancy system which is used by the majority of the inhabitants of the lines, although some of the people make use of a piece of waste land near the lines, for purposes of defecation. In the mill itself there is a system of septic tanks and water carriage of sewage, used while the employees are at work. In section 2 there is an imperfect and badly supervised pan system, and many of the villagers defecate in the adjoining field. Section 3 is situated on the bank of a large tank and there are no latrines, defecation being performed on the bank of the tank opposite the village.

Practically two hundred stools were collected from each section every month for a period of twelve months. It was impracticable to collect from the same individuals on each occasion, because most of the coolies are only engaged on three months' contracts at the mill, so that there is a constantly changing population.

The inhabitants of the area coming as they do for short employment from a wide area of the surrounding country, the figures obtained in this investigation are probably of general application to the rural districts of Bengal

Stools handed in on the morning they were passed, to one of my assistants were placed in measured quantities in antiformin solution in the same manner as described in my earlier work (Maplestone, 1929). They were brought to Calcutta and the counts after Stoll's method were made in my laboratory by the same workers who assisted me on former occasions. In the table given below the percentage of infected cases has been determined by the use of Lane's centrifuge, because samples of all the stools were subjected to this method of examination before being diluted for counting. This saved a good deal of time as stools negative to 'D C F' were not counted.

TABLE I

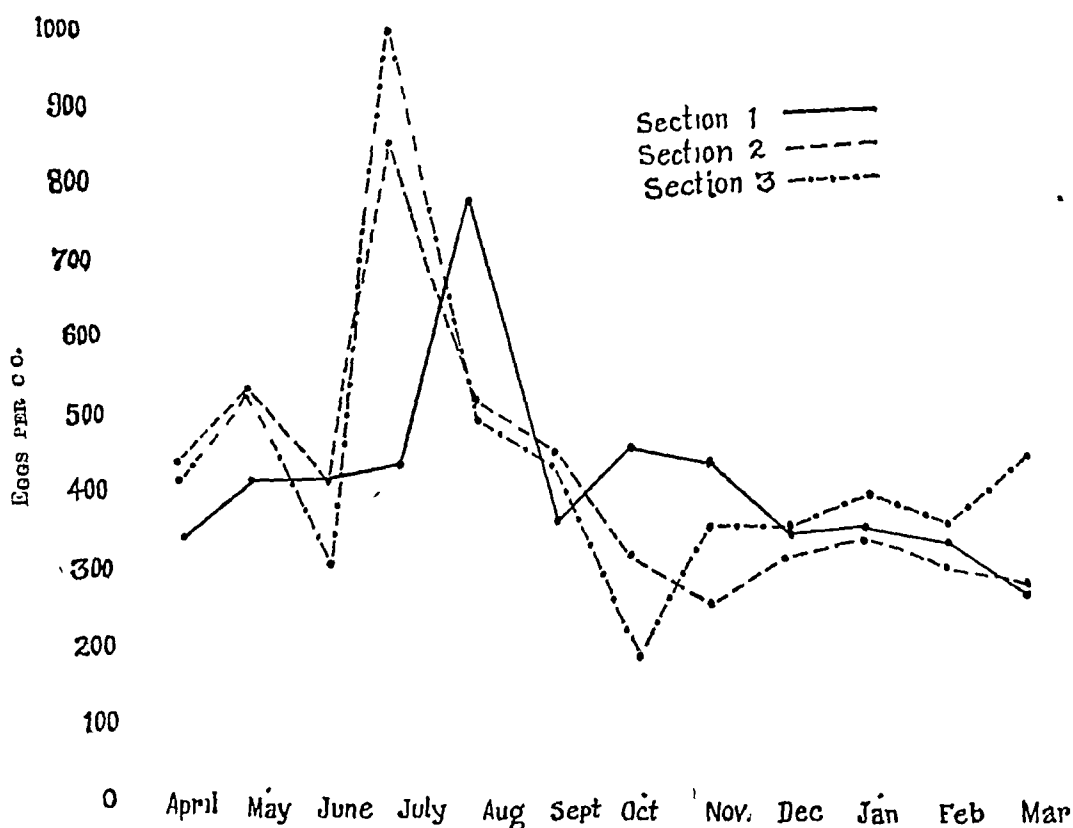
Number of cases examined, percentage positive for hookworm infection, and the average number of eggs per c.c. of the total examined

MONTH.	SECTION 1			SECTION 2			SECTION 3		
	Number examined	Percentage infected	Average eggs per c.c.	Number examined	Percentage infected	Average eggs per c.c.	Number examined	Percentage infected	Average eggs per c.c.
April	200	70.5	340	200	74	436	198	75.5	409
May	200	77	113	198	80	533	198	79.5	532
June	200	80.5	418	200	81	413	200	83	303
July	170	80.5	434	193	75	841	200	85	986
August	199	82.5	773	198	83.5	509	200	82.5	493
September	200	80	358	200	77	445	198	91	431
October	199	83	454	199	85.5	309	196	77	186
November	197	73	453	200	70	252	200	64.5	354
December	200	80	340	200	82.5	313	200	80.5	344
January	200	79.5	343	200	73	336	199	75	382
February	200	78.5	323	200	75.5	290	200	82	345
March	200	78	273	200	69	264	199	76.5	433

In Chart 1 it will be seen that two of the series reach their peaks in July, and the other one is at its highest in August. This is not regarded as of any significance, because the counts being made at monthly intervals, this period of time will represent the possible error in determining the actual highest point in the egg counts, i.e., the maximum for sections 2 and 3 may not have been reached by July, and the maximum for section 1 may have been passed by August, and if this is the

CHART 1

Average monthly egg counts for each of the three sections shown separately



case more frequent counts between July and August would have brought the three peaks closer together. It will also be noted from Chart 1 that the effect of the better sanitary conditions prevailing in section 1 are not very apparent, this is probably because the population is continually changing and so new arrivals come in to keep up the average. However, as far as it goes the difference between the charts for this section and sections 2 and 3 is suggestive, for the maximum infection rate is lower in this than in the less sanitary areas.

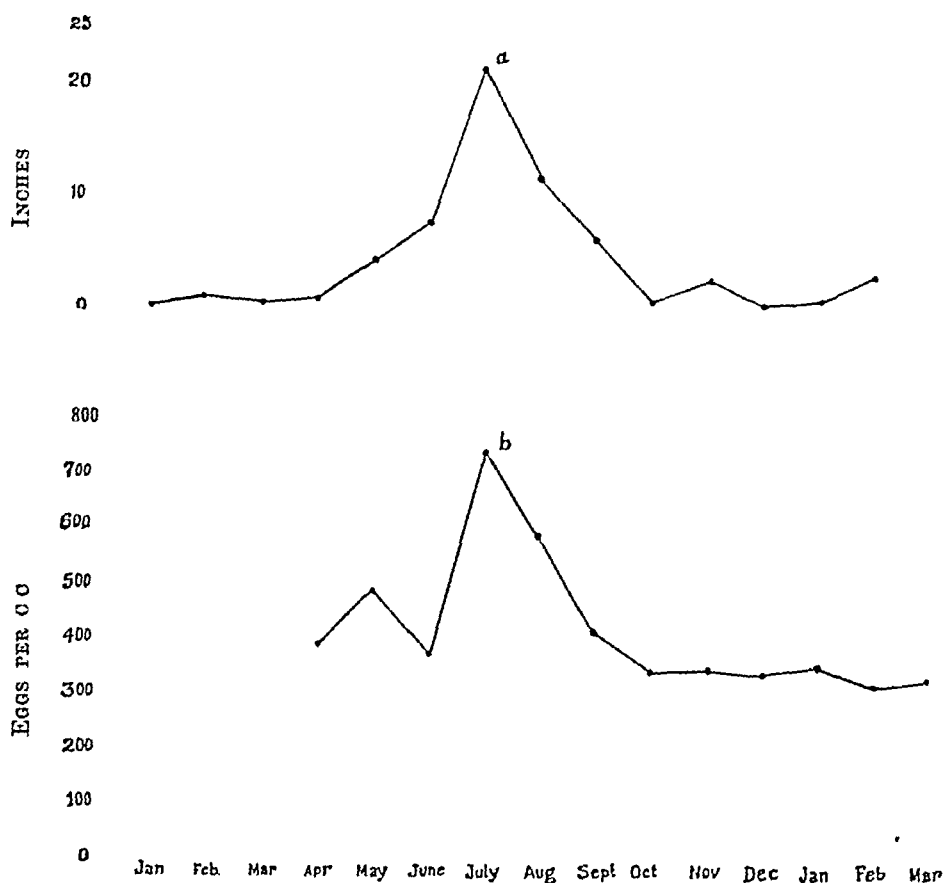
1148 *Observations on Seasonal Variation in Hookworm Infection*

Chart 2, which shows the average of the three counts combined and the monthly rainfall for the time of the examination, is remarkably like that of the chart for the two Sylhet gardens given in my former paper in which monthly counts were also done

In the present instance it will be clear from the table that the percentage of infected persons did not vary very much from month to month and it is quite without significance

CHART 2

- (a) *Monthly rainfall in inches during the period of the investigation*
 (b) *Average monthly egg counts of the three sections combined*



Ascaris infection varied between 33 per cent and 75 per cent with an average of 15.7 per cent, for the whole thirty-six monthly counts made, and *Trichuris* infection varied between 33 per cent and 4 per cent with an average of 17.3 per cent. These figures are much too small on which to base any conclusions, except that there was no sign of correlation between the variation noted and the rainfall, so

they have not been included In my former paper with much higher infection rates with these two worms no connexion between the egg counts and the rainfall could be discovered either

Two papers have recently appeared in American journals on the rate of loss of hookworm infection In the first of these the Caldwells (1931) studied the inmates of an institution where re-infection was impossible, and the members of a rural community during a period unfavourable for infection to occur They also kept under observation for a period of four years a case of laboratory infection, and the results were the same in all three series, viz, there was no apparent loss of infection as measured by egg counts In the second paper the Paynes (1931) examined three cases very frequently for periods varying from about five weeks in the shortest to three months in the longest, and they found no significant difference in infection rate throughout

From my own experience of a few cases in which I have been able to exclude the possibility of re-infection, such as that of Europeans on leave in England for anything up to one year, and returning to India with hookworm infection, I am in agreement with the findings of the above workers In spite of these facts there seems to be no doubt from my work, embracing as it does four widely separated areas in Bengal and extending over a period of about three and a half years, that in this portion of India at all events there is a distinct annual variation in egg counts, which presumably indicates a variation in the number of worms per head of the population, and that this variation is in close association with the rainfall, but in other parts of the world where investigations in any way resembling those done in Bengal have been made, no such variation has been discovered The reason for this change has already been discussed in detail in my earlier paper, so it is not proposed to repeat it in this one, but why this change is so rapid and the increase so short-lived is not yet explained I recently had the opportunity of discussing the matter with Dr W C Sweet of the Rockefeller Foundation, and he suggested, though with great diffidence, that the extra worms the people apparently pick up during the April-June period fail to become properly established, and from some unfavourable factor which is not at present obvious they die off as rapidly as they are acquired, leaving the infection at its old level There is another possible explanation, and that is that for some reason or other hookworms lay more eggs during the early monsoon period than during the rest of the year This idea was suggested by Dr J B McVail some years ago before the days of 'D C F' and egg counts, to explain why more infections were found during the monsoon than in the dry season, and that the worms were inclined to lay more freely when the conditions were more favourable for development of larvæ There are two points against this, however, the first is that worms living inside an intestine would hardly be in a position in which they could appreciate alteration in meteorological conditions, and the other is that if the worms actually had this power they would not

decrease their egg-laying at the height of the monsoon as they appear to do from my charts

The writer is aware that neither of the above suggestions are probably of much value but they are put forward in the hope that others may be encouraged to consider the point and perhaps suggest something better

In my former paper attention was drawn to the marked effect differences in sites of coolie lines in the Sylhet gardens had on the degree of infection by intestinal nematodes. In the Dooars last year the opportunity arose of collecting stools from coolie lines about ten miles from where the previous prolonged investigation was carried out. The average of the two counts made in June 1928 and June 1929 are given in the table below with the average of two hundred stools collected in the other garden in July 1930

TABLE II.

Percentage infected and average number of eggs per c c for total examined in two tea garden areas in the same district. In one the soil is favourable for hookworm development, in the other unfavourable

Year examined	HOOKWORM		ASCARIS		TRICHURIS	
	Percentage positive	Average eggs per c c	Percentage positive	Average eggs per c c	Percentage positive	Average eggs per c c
1928 and 1929	93.9	1,125	87.5	6,342	79	286
1930	59	148	27	719	28.5	5

It is considered that the figures obtained in 1928 and 1929 may be compared with those from a different garden in 1930 for the times that they were collected are within a month of each other in the three years, and the seasons of all three years were closely similar as regards onset and amount of rainfall

It is evident that in the garden examined in 1930 the worm infection is negligible. In neither of these places is anthelmintic treatment carried out as a routine, and the coolies on all gardens are of similar castes and habits, the only difference is in the nature of the soil on which the lines are placed. In the first gardens where the worm infection rate is relatively high the lines are surrounded by sandy soil which rapidly drains off excess of moisture but remains moist under the grass for a long

time In the second garden the lines are surrounded by heavy red soil of clay-like consistency, which holds water a long time and when it finally dries becomes extremely hard

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RESEARCHES ON 'STONE'.

Part XIII.

X-RAY DIFFRACTION STUDIES OF CALCULI

BY

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[Received for publication, December 28, 1931]

Abstract.

THE paper describes the X-ray diffraction studies made of vesical calculi (human and cattle), gall stones from human beings, uric acid and cholesterol. It is to be observed, in general, that in all the varieties of stones examined, the substance or substances that constitute the calculi are deposited in the crystalline state, though a little variation in the order of magnitude of the crystals was observed. For instance, in the pigmented gall stone, cholesterol appears to be deposited in a micro-crystalline state as is evidenced by the contraction of the rings and by the absorption of the bile pigments. The diffraction patterns obtained by vesical calculus (human) and pure uric acid show several features in common, estimations of the spacings of the corresponding rings in both gave identical values. The rings, characteristic of the components of the stone other than uric acid, were not present on account of insufficiency of exposure. Gall stones present several interesting phenomena, chief of which are the occurrence of intensity maxima arcs and the contraction of the rings characteristic of cholesterol. The former is analogous to the phenomenon occurring in pressed paraffin wax and stretched gelatin where there is orientation of the crystals in certain preferential directions, and the latter is attributed to the extremely fine state of division of the crystals. An explanation has been offered, in the light of the X-ray analysis, for the existence of the two varieties of gall stones, the pigmented and the non-pigmented. Uric acid in human stones and calcium carbonate in cattle stones are definitely shown to exist in the crystalline state.

Introduction.

It is well known that calculi are concretions formed under certain pathological conditions inside the urinary bladder, kidney, gall bladder, etc. Comparatively greater work has been done on urinary calculi—bladder and kidney—than on biliary calculi. It is believed that in urinary calculus formation the colloids present in the urine play an important rôle. The theories proposed to explain the function of the urinary colloids are many, chief of them being the 'protective' mechanism attributed to the colloids, by virtue of which the sparingly soluble substances present in the urine, like uric acid and urates, oxalates and phosphates of calcium, etc., are not only held in high concentration but also excreted in the urine in a non-coalescent form. The 'protective' colloids are believed to enmesh the crystalline particles by forming a thin film around them, or, it might just be possible that in the presence of these 'protective' colloids, the sparingly soluble substances exist in the urine in a colloidal state and in pathological conditions get deposited as such, finally resulting in the hard stone. A similar phenomenon is stated to occur in gall stone formation.

The state in which the substance or substances that make up the calculi are deposited has not been known with any certainty. So far, only chemical and physico-chemical methods have been attempted, but the results obtained by different investigators were not consistent. The above methods of investigation are but crude before purely physical methods and, of these, *X-ray* diffraction studies afford a more definite method of deciding whether the substances in the stones are deposited in the colloidal or crystalloidal state. It is already known that crystals in a finely powdered state give rise to sharp and well-defined rings while colloidal substances give diffuse bands (Clark, 1927*a*), crystals not powdered will give numerous 'Laue' spots. Hence the present investigation was undertaken. This is the first attempt, so far as the author is aware, to study calculi by means of the *X-ray*. The results obtained are encouraging.

Methods of Investigation

The stones examined were (1) human vesical calculus, (2) vesical calculus from cattle, (3) human gall stones, (4) uric acid, and (5) cholesterol. A thin section of the substance, about quarter to one millimetre thick was used, depending on the nature of the substance. The above were examined both in their original as well as in the powdered state.

Gall stones vary in their chemical composition, but they are believed to contain about 90 per cent of cholesterol. With a view to comparing the diffraction pictures obtained from gall stones with that of pure cholesterol, the latter was tried. To obtain the powder pattern, cholesterol was very finely powdered in an agate mortar and pestle. Likewise, pure uric acid was also tried.

with a view to comparing the pattern obtained with that of a stone containing uric acid

To get the X-ray patterns, the pin-hole method of Debye and Scherrer was employed. A detachable Shearer X-ray tube with a copper target served as the source of the radiation. By the use of a thin aluminium window, the X-rays were made to pass without excessive absorption. The tube was worked by an oil-cooled transformer at about 50 K V and a fairly steady current of 4 to 5 milliamperes was maintained in the tube. The radiation emitted consisted of $K\alpha$ and $K\beta$ of copper, but since the latter was of feeble intensity, a filter was not employed. The distance between the substance and the photographic plate was 2.64 cms. The exposures varied from three to ten hours depending on the nature of the substance. Iso-zenith plates (700 H D) were used. The crystal spacing corresponding to any particular halo of angular radius, θ , is calculated from the well-known Bragg formula,

$$d = \frac{\lambda}{2 \sin \frac{\theta}{2}}$$

where d is the intermolecular spacing and λ the wave-length of the incident X-rays (here the value being 1.54 \AA) due to Cu $K\alpha$. The negatives were examined visually and special attention was paid to the sharpness or diffuseness of the maxima as well as to any general scattering present.

Results.

Gall stones The gall stones examined were obtained from the Pathological Museum of the Medical College, Calcutta, through the kindness of Dr. Sur, Professor of Pathology to whom the author is indebted.

There are commonly two varieties of gall stones, the pigmented and the non-pigmented. They are usually supposed to consist for the most part of cholesterol with small amounts of bilirubin either as such or admixed with calcium as calcium bilirubinate. The pigmented variety investigated was a beautifully striated stone of several concentric layers around a central nucleus. The outside of the stone was light coloured, on cutting a section through the stone, the inside presented a darker shade of the pigment. Unlike the pigmented stone, the non-pigmented stone appeared glistening even on the outside and on cutting it open the glistening of the crystals was all the more apparent. It looked as though it were an aggregate of loosely bound crystals, it was not possible to have a thin section of the stone cut. The crystalline character of the stone was obvious even from naked-eye observation.

Pigmented stone One millimetre-thick section of the stone was used to obtain the X-ray diffraction pattern. It gave three well-defined rings, two of which were sharp with still sharper one between (Plate LVII, fig. 1). The radii and spacings calculated therefrom are given in Table I.

Besides these rings, an interesting phenomenon observed was that there were two dark patches in the ring one above the other, the two being separated by a distance equal to the diameter of the ring. To ascertain the relative position of these intense maxima with reference to the striations in the stone, the experiment was repeated with the striations running parallel to one another, i.e., lying in the horizontal position. The diameter of the ring joining the two patches was perpendicular to the striations.

Non-pigmented stone One millimetre-thick section of the stone was used. It gave two faint rings almost corresponding to those of the pigmented stone (Plate LVII, fig 2). The central sharp ring was not observed. In addition to these rings, numerous 'Laue' spots were obtained. The intense maxima arcs observed in the inner ring of the pigmented stone were not present here. The radii of the rings and the spacings are given in Table I.

Cholesterol The substance used was Schering-Khalbaum's. One millimetre-thick of the very finely powdered substance was used. It gave two rings and the most interesting point about them was that their size appeared even from naked-eye observations to be slightly larger than those obtained with the two varieties of gall stones (Plate LVII, fig 3). Calculations of the spacings revealed the same difference, as is shown in Table I —

TABLE I
Cholesterol and cholesterol stones

Particulars		I ring	II ring	III ring	REMARKS
Pigmented gall stones	Inner radius	6.2 mm	10.3 mm	11.5 mm	Two intense maxima arcs in the I ring. II ring was very sharp. I and III were also sharp.
	Outer radius	8.4 "		13.0 "	
	Mean radius	7.3 "	10.3 mm	12.25 "	
	Spacing (d)	5.72 A°	4.16 A°	3.57 A°	
Non pigmented gall stones	Inner radius	6.0 mm		11.5 mm	The middle ring obtained above was not observed here. Rings were sharp.
	Outer radius	8.4 "		12.2 "	
	Mean radius	7.2 "		11.85 "	
	Spacing (d)	5.79 A°		3.68 A°	
Cholesterol	Inner radius	6.5 mm		12.0 mm	Sharp rings
	Outer radius	8.8 "		13.2 "	
	Mean radius	7.65 "		12.6 "	
	Spacing (d)	5.48 A°		3.49 A°	

Vesical calculus (human) The stone was obtained from Peshawar, North-West Frontier Province, and was one from Colonel McCarrison's collection. It was a fairly hard, egg-shaped, whitish stone weighing about a hundred grammes. On sectioning it through its centre by its shorter axis with a hack-saw, it was observed that the stone was made up of a central, hard, dark-coloured nucleus, surrounded by a broad, yellowish layer of uric acid, then alternating layers of phosphates and urates, and finally an external whitish layer of magnesium ammonium phosphate. A fairly representative sample of the stone was used in its finely powdered form, the thickness employed being above half-a-millimetre. The X-ray diffraction pattern showed two sharp rings (Plate LVII, fig 4). The radii of the rings and the intermolecular spacings calculated therefrom are given in Table II.

Uric acid Since it was believed that the exposure given for the above stone was not enough to excite the ash rings and that the diffraction pattern was derived mostly from its uric acid and urate constituents, pure uric acid (Khalbaum's) was tried in its very finely powdered state. It gave rise to four rings, only one of which was very bright, the rest were comparatively faint, the outermost ring being especially so (Plate LVII, fig 5). The radii and spacings calculated therefrom are recorded below in Table II —

TABLE II

Vesical calculus (human) and pure uric acid

Particulars		I ring	II ring	III ring	IV ring	REMARKS
Vesical calculus (human)	Inner radius	6.8 mm		13.3 mm		Sharp rings
	Outer radius	8.2 "		15.0 "		
	Mean radius	7.5 "		14.15 "		
	Spacing (d)	5.58 Å°		3.15 Å°		
Uric acid	Inner radius	6.0 mm	10.3 mm	13.3 mm	22.4 mm	I ring very intense and broad, the others comparatively faint, but not diffuse
	Outer radius	9.0 "	11.5 "	15.5 "	23.5 "	
	Mean radius	7.5 "	10.9 "	14.4 "	22.95 "	
	Spacing (d)	5.58 Å°	3.96 Å°	3.11 Å°	2.20 Å°	

Vesical calculus (cattle) It was obtained from Kistna District, Madras Presidency, from a bullock ten years old, it was one from Colonel McCarrison's collection. Unlike human stones, cattle stones are usually small, grain-like bodies often little bigger than a No. 4 shot, having a light deep golden yellow sheen. They have a laminated structure of several layers all looking alike and having the

same composition, the layers being thin and comparable to the thin coats of the onion

A thin section of the stone was exposed for about twelve hours when four faint but sharp ash rings at large angles were obtained. The radii and spacings are recorded in Table III —

TABLE III

Vesical calculi (cattle)

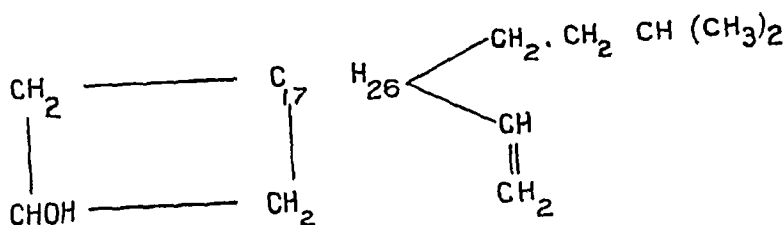
Particulars	I ring	II ring	III ring	IV ring
Radius	16.5 mm	18.8 mm	22.5 mm	25.3 mm
Spacing (d)	2.79 Å	2.10 Å	2.23 Å	2.07 Å

Discussion

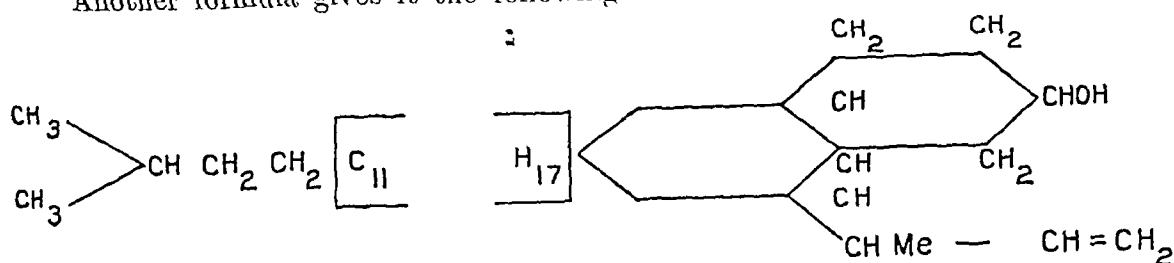
Cholesterol and cholesterol stones Comparing the X-ray diffraction patterns obtained from the two types of gall stones with those due to pure cholesterol, it is seen that in the pigmented variety, the rings were as sharp as those of powdered crystalline cholesterol, showing thereby that the substance is deposited in a micro-crystalline state, almost bordering on colloidal dimensions, while the non-pigmented variety gave rise to both rings and 'Laue' spots. The 'Laue' spots indicate bigger crystals. In both the instances, the rings were quite sharp. In addition to the rings, there were two intensity maxima arcs observed in the pigmented stone in a plane perpendicular to the striations of the stone. Possibly there are two cases, one in which all the crystallites are randomly distributed and the other in which the crystallites have been given orientation in certain preferential directions. The origin of this orientation is presumably due either to compression or to the method of deposition itself. This phenomenon bears a close analogy to that occurring in pressed paraffin wax (Clark, 1927b) and stretched gelatin (Clark, 1927c), but the orientation is not so perfect. Accordingly, arcs of intensity maxima rather than definite maxima points are produced in the Debye-Scherrer pattern. The diffraction patterns obtained show, in general, that the substance has been given a fibrous structure.

Cholesterol, the chief ingredient of gall stones, is chemically a complex molecule with several side chains. The formula shows it to be fairly elongated. One formula, shown below, has it as a polycyclic, secondary ring alcohol, with several

side chains among which isoamyl and vinyl group are to be identified, the latter giving opportunity for further ring formation (Richter, 1922) —



Another formula gives it the following structure —



The cholesterol monohydrate, $C_{27}H_{45}OH$, H_2O , belongs to the trichinic pinakoidal system, $a = 0.375$, $b = 0.396$, $c = 0.396$, $\alpha = 90^\circ$ Ca, $\beta = 100^\circ 30'$, $\gamma = 90^\circ$ Ca (Groth, 1910)

Comparing the diffraction patterns of the gall stones with that of pure cholesterol, it is seen, as remarked previously, that the rings in the stones are smaller. This may be due either to a structural re-arrangement in the cholesterol molecule itself, possibly in its side chains, or more likely to a finer state of division of the cholesterol crystals in gall stones. It is well known that cholesterol undergoes reduction in the intestine to Coprosterol, $C_{27}H_{47}OH$ (?) (Richter, *loc cit*). But it is more likely that the contraction of the rings may be due to a finer state of division. Lowry and Bozorth (1928) have obtained a similar contraction of rings in graphitic charcoal prepared in a very fine state of division. Krishnamurthi (1930) obtains for sugar charcoal a spacing of 3.8 \AA which represents a much finer state of division than that of Lowry and Bozorth. The latter also observe that this fine state of division is associated with adsorptive properties. The adsorption of bile pigments in the pigmented gall stone affords confirmative evidence for the extremely fine state of division of the cholesterol crystals. The absence of such adsorption in the non-pigmented stone is probably due to the bigger size of the crystals as revealed by the numerous 'Laue' spots, though in addition to them rings were obtained. The preponderance of the bigger size crystals perhaps masks the adsorptive properties of the small amount of cholesterol in a fine state of division, the existence of which even in the non-pigmented stone is not denied.

Sweet (1930) observes that in the formation of gall stones, there is a shift of the cholesterol from the colloidal to the crystalloidal state. Whatever the antecedent state of cholesterol in body-fluids may be, X-ray analysis shows that it is deposited in gall stones in the crystalline condition.

Vesical calculus (human) and uric acid The diffraction patterns obtained for the vesical calculus and uric acid showed some interesting features in common. Pure uric acid gave rise to four rings, the first ring being made up of two rings touching each other, the second ring very sharp but of feeble intensity, the third very intense and comparatively broad, and the fourth very faint and sharp. Compared to the outer edge of the first ring the inner edge was fainter. The stone investigated was composed of uric acid and urates, magnesium ammonium phosphate and calcium phosphate, it contained a good percentage of each. This stone gave rise to only two rings. The innermost ring in the uric acid pattern was very broad and faint whereas in the stone pattern it was quite sharp and narrow. The latter did not seem to be made up of two rings. But the mean radii of the rings in both were exactly the same. The second ring seen in the uric acid pattern was not observed in the stone pattern. The third ring was quite sharp, yet its intensity was not as much as that in uric acid, and hence probably, the second and the fourth, the two comparatively faint rings, did not make their appearance.

The spacings expressed in Angstrom units, of the two rings obtained with the stone show unmistakably that they correspond to the uric acid component of the stone. Though the stone contained, besides the uric acid, a good percentage of magnesium ammonium phosphate and a little calcium phosphate—components containing elements of comparatively higher atomic weight—possibly on account of insufficiency of exposure, the ash rings were not excited. But the one definite result arrived at from X-ray study of the vesical calculus is that its uric acid component is deposited in a crystalline state, as shown by the identity of spacings of the rings obtained both with the stone and with pure uric acid. Schade and Boden (1913) believe that uric acid is held in the urine in its colloidal state while Lichtwitz (1913) and Gudzent (1914) are opposed to the existence of a such colloidal state. Haskins (1916) is inclined to believe that at least a part of the uric acid is held in the colloidal state. As the state of uric acid in the stone is likely to give a clue to the state of its existence in the urine, it is assumed on *a priori* considerations that the uric acid exists in the urine in a crystalline state.

Vesical calculus (cattle) The cattle stone examined was made up for the most part of calcium carbonate, chemical analysis showed it to contain moisture, 3.5 per cent, ash, 54.2, total nitrogen, 0.4, P_2O_5 , trace, CaO, 44.4, MgO 9.4, and CO_2 , 38.4, all figures excepting that for moisture represent percentages on moisture-free stone (Ranganathan, 1931). The sharp rings obtained in the diffraction pattern show that the substance is deposited in the crystalline state. The absence of uric acid in cattle stones is also indicated in the X-ray analysis.



Fig 1—X ray diffraction pattern of human gall stone pigmented variety

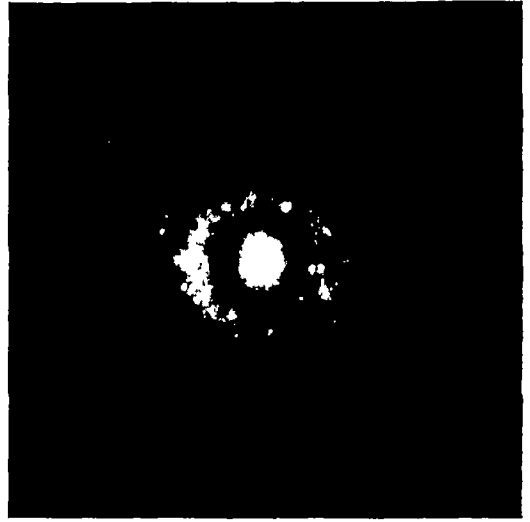


Fig 2—X ray diffraction pattern of human gall stone non pigmented variety



Fig 3—X-ray diffraction pattern of cholesterol



Fig 4—X-ray diffraction pattern of vesical calculus (human)



Fig 5—X-ray diffraction pattern of pure uric acid

In conclusion, the author desires to express his most grateful thanks to Professor Sir C V Raman, F R S, for his inspiring guidance and his keen interest in the progress of this work. The investigation was carried out in the laboratories of the Indian Association for the Cultivation of Science, Calcutta, and forms part of the researches on 'stone' conducted under the direction of Colonel McCarrison.

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THE INFECTION OF STRATIFIED EPITHELIUM IN LEPROSY.

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[Received for publication, January 16, 1932]

It is generally supposed that dermal infection with *M lepræ* is confined to the corium and that the stratified epithelium of the skin escapes infection and thus acts as a protective covering preventing the organisms escaping from the body and infecting contacts

We had always held that the above view is correct except in severe cases of lepra reaction in which the epithelium is not entirely intact and *M lepræ* can be recovered from scrapings of the epithelium

The following case has, however, lead us to modify our view —

Dhruv (see Plate LVIII, figs 1 and 2) was at first treated at the skin clinic as a case of ichthyosis. Later an anæsthetic patch was found on one of his feet and he was sent to the leprosy department as a suspicious case of leprosy. There was nothing in the appearance of the patient to lead one to suppose that he was a case of leprosy—still less that he was an advanced cutaneous case, but clip-smears taken from various parts of the body all showed large numbers of Hansen's bacilli. There was not a sign of a nodule anywhere on the surface of the body and diffuse swelling, though present to a certain extent, was hardly noticeable. Sections were cut of the skin and abundant acid-fast organisms were found especially in the more superficial parts of the corium and chiefly in and around the capillaries. The papillæ were not flattened out to the extent generally found in this type of cutaneous

leprosy Round the blood vessels there was small cell infiltration with a certain number of large epithelioid cells, but beyond the perivascular areas the elastic fibres were intact and no infiltration had taken place The vascular plexus in the papillæ was picked out with acid-fast organisms almost as if it had been injected with them They were contained chiefly within the endothelial cells, though a certain number had escaped from cells probably due to the process of sectioning But the remarkable thing was that large, round clumps of acid-fast organisms were found in the malpighian layer of the epithelium and flattened clumps were present lying between the flattened, more superficial epithelial layers and also inside the loosened scales on the surface (Plate LIX, fig 3)

It was supposed at first that some of these organisms had been displaced by the microtome knife and carried on to the epithelium from the corium, but examination of other sections proved that this was not so, but that clumps of *M lepræ* had actually entered the deeper layers of the epithelium and been gradually carried to the surface by the cells, the clumps being moulded to the shape of the successive layers of epithelial cells as they passed outwards

Superficial scrapings of the epithelium were taken with a knife from various parts of the body, only the superficial scales being removed In several of these the scales were found to contain abundant acid-fast bacilli both singly and in clumps (Plate LX, fig 4)

This is an important finding from the point of view of the dissemination of the disease *M lepræ* encased in epithelial scales were constantly being shed from the whole surface of the patient's body We have no direct proof that these organisms are alive, cultural and experimental evidence not being available, yet the fact that they were uniformly stained and had remained encased in epithelial cells without a chance of desiccation is in favour of their being alive and therefore a potent agency in spreading infection

Subsequent scrapings taken from other patients with intact epithelium have in several cases shown similar findings

It must therefore be recognized that unbroken epithelium does not always prevent the escape of *M lepræ* from the surface of the body, as in some cases these organisms are shed from the surface of the skin either free or contained within scales of epithelium

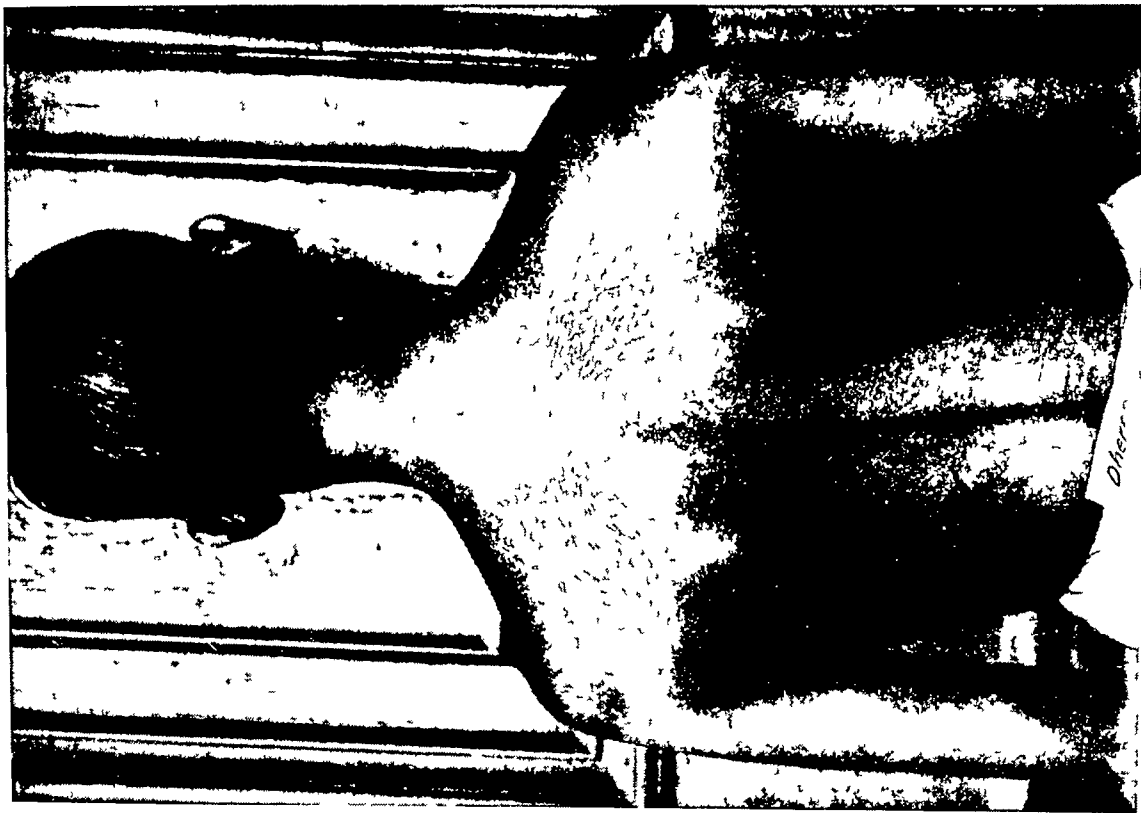


Fig. 1

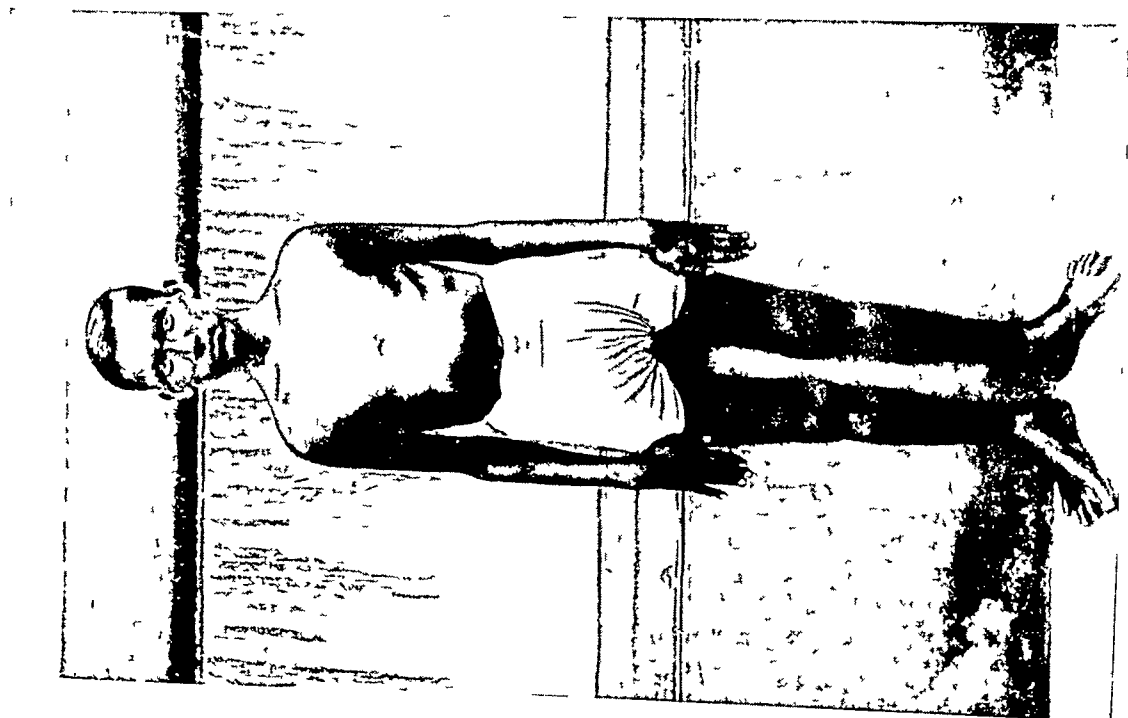


Fig. 1

PLATE LIX

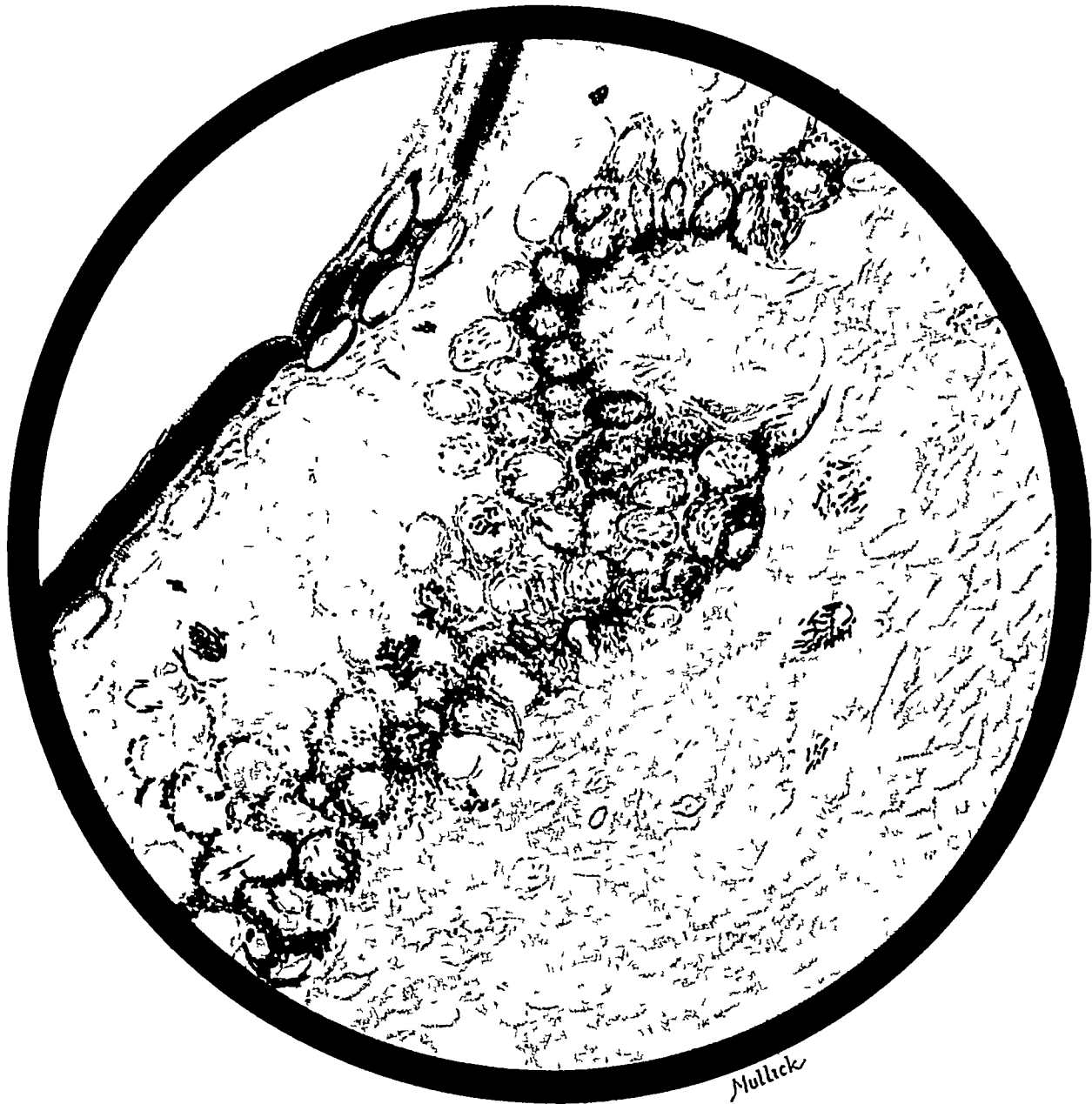


Fig 3 —Section of Skin Leprosy showing *M lepræ* in clumps and singly in Epithelium

PLATE LX

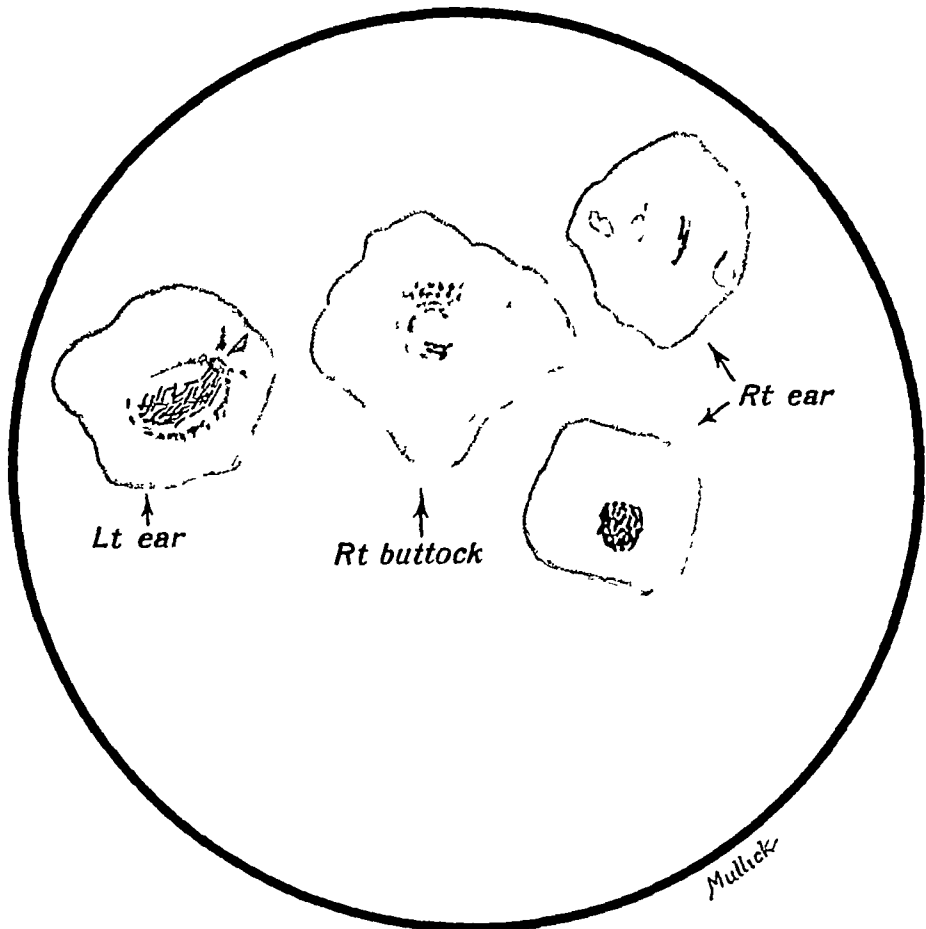


Fig 4 —Epithelial scales from skin of different parts of body containing *M. lepræ*

THE VITAL CAPACITY OF 103 MALE MEDICAL STUDENTS IN SOUTH INDIA

BY

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[Received for publication, January 16, 1932]

As the vital capacity test is not only of physiological importance but is also of valuable aid in the diagnosis of certain diseases, chiefly of the heart and the lungs, and in the guidance of treatment of such diseases, and as the vital capacity standards laid down in the Western countries are not applicable to the people in a tropical country like India, it is necessary that vital capacity estimations should be made in different parts of the country and standards be laid down exclusively for Indians as in the case of every other physiological standard

Dreyer (1919) pointed out that various races of people would probably be found to have different normal vital capacities. Wilson and Edwards (1921) found the vital capacity of 38 coloured children of 8 to 14 years of age to be strikingly below the western standards and suggested a possible racial factor. Foster and Hsieh (1923) examined 500 Chinese including women and found the vital capacity in men to be 2.0 litres and women 1.5 litres per square metre of body surface as compared with 2.5 litres in men and 2.0 litres in women in America. Myers found, in small groups of Chinese and Philipinos, the vital capacity to be lower than among the white races.

So far, very few estimations of vital capacity seem to have been done in India. In 1929 Bhatia, in a paper read at the 16th Indian Science Congress, pointed out that the vital capacity in 100 Indians at Bombay was much smaller than that of Western people. H. N. Mukherjee and P. C. Gupta in a paper on basal metabolism, published in the *Indian Journal of Medical Research*, **18**, No. 3, January 1931, referred to their observation that the vital capacity of 12 subjects averaged 2.225 litres per square metre (deviation of —14.8 per cent) and stated

that according to the report on the 'students welfare scheme' of the University of Calcutta, the average vital capacity of Bengali male students was even lower than their average

With a view to find the standards in South India, the vital capacity tests were made on 103 male medical students ranging from 18 to 29 years coming from different parts of the Presidency. The dry type of spirometer was used for estimations. Three trials were allowed in each case and the highest reading was recorded as the vital capacity. Certain measurements of the body such as standing height, sitting height, weight, body surface area and chest circumference were also recorded. The body surface area was calculated from the charts devised by Aub-DuBois, based on height-weight formula. Table I represents the vital capacities and the measurements such as standing height, weight, and body surface, of 103 subjects of 18 to 29 years of age. Table II represents the vital capacities of 58 subjects of 18 to 25 years of age included in Table I and their sitting heights and chest measurements.

The average vital capacity of all the subjects is found to be 2.29 litres and the average per square metre of body surface area to be 1.85 litres (Table III).

The correlation between the vital capacity and certain measurements of the body such as standing height, weight, body surface, etc., has been shown by workers in the West to be fairly constant.

Age—It was Wintrich (1854) who assigned to age a great influence upon the vital capacity. He found the largest vital capacities in persons between the ages of 20 and 40. Hutchinson (1846) had found the maximum to be at the 30th year. More recently, Stewart (1922) found the maximum in boys to be at the 20th year and in the girls slightly earlier. In the series of cases I examined, the range of age is too small to dogmatize about the influence of age, but, judging from the results recorded, there seems to be a gradual rise from the 18th year onwards up to the 21st year when the maximum is reached and then a decline (see Table III and Fig. 1).

TABLE I
Showing vital capacity of medical students

Number	Age	Race	Height, cm	Weight, kilo	Body surface, sq. m	Vital capacity, c.c.	Vital capacity per sq. m, c.c.
18	18	H. Nb	167	52.5	1.59	2,658	1,670
108	18	H. Br	166	57.0	1.63	3,248	1,995
109	18	I. C	152	36.5	1.29	2,000	1,573
43	18	H. Br	172	54.0	1.63	2,660	1,610

TABLE I—*contd*

Number	Age	Race	Height, cm	Weight, kilos	Body surface, sq m.	Vital capacity, c c	Vital capacity per sq m., c c
9	19	H Br	175	46 0	1 55	3,156	1,897
43	19	H Br	156	52 2	1 48	2,658	1,790
4	19	H Nb	163	48 0	1 49	3,642	2,442
19	19	H Br	166	54 1	1 59	2,790	1,755
20	19	H Br	178	45 4	1 56	2,772	1,775
42	19	H Br	170	49 1	1 56	2,160	1,578
22	19	H Br	163	48 5	1 50	2,790	1,862
66	19	H Nb	160	50 8	1 51	2,298	1,520
1	20	H Nb	170	56 2	1 64	3,690	2,250
38	20	S C	164	56 0	1 60	2,460	1,546
65	20	H Br	167	50 2	1 56	2,956	1,892
28	20	A I	169	50 8	1 58	3,442	2,180
29	20	A I	177	52 7	1 64	3,610	2,200
37	20	A I	159	44 5	1 42	3,200	2,258
47	20	H Nb	174	53 2	1 58	2,460	1,560
87	20	H Nb	171	54 0	1 63	3,280	2,010
15	20	H Br	171	60 4	1 70	2,694	1,582
3	20	H Nb	160	54 5	1 55	2,658	1,710
7	20	H Nb	165	59 0	1 61	3,150	1,955
25	20	H Br	168	45 9	1 50	2,886	1,925
30	20	A I	162	57 3	1 60	3,398	2,120
49	20	H Br	162	54 5	1 56	2,740	1,753
53	20	H Br	168	52 7	1 60	2,266	1,420
57	20	H Nb	173	48 8	1 58	3,280	2,076
64	20	H Nb	165	47 7	1 51	2,298	1,520
79	20	Jam	170	45 0	1 50	1,968	1,312

TABLE I—*contd*

Number	Age	Race	Height, cm	Weight, kilos	Body surface, sq m	Vital capacity, c c	Vital capacity per sq m, c c
26	21	H Nb	161	43.4	1.42	2,790	1,965
17	21	H Br	166	44.3	1.46	2,740	1,880
16	21	H Nb	166	53.0	1.58	2,758	1,742
56	21	S C	167	54.0	1.61	2,330	1,448
31	21	A I	185	72.0	1.95	4,640	2,380
10	21	S C	164	54.0	1.58	4,150	2,652
8	21	S C	159	51.3	1.52	2,956	1,942
23	21	H Nb	164	48.7	1.51	2,920	1,932
78	21	H Nb	170	53.1	1.61	3,280	2,040
105	21	H Nb	163	49.6	1.52	2,972	1,890
100	21	I C	168	45.4	1.50	2,460	1,640
32	21	H Br	171	69.5	1.81	4,130	2,284
33	21	A I	169	54.2	1.61	3,858	2,400
74	21	H Br	168	62.3	1.70	3,510	2,062
81	21	H Nb	165	42.3	1.43	2,542	1,780
103	21	H Nb	155	44.5	1.40	2,874	2,054
55	22	S C	165	48.8	1.52	2,460	1,619
39	22	H Br	166	55.7	1.62	2,166	1,340
85	22	H Br	166	50.7	1.56	3,524	2,260
73	22	H Nb	170	54.8	1.63	2,956	1,810
38	22	H Br	165	50.8	1.55	2,460	1,588
11	22	S C	159	45.4	1.44	3,082	2,142
61	22	H Br	172	47.3	1.54	3,138	2,034
112	22	H Br	172	56.8	1.66	3,200	1,925
83	22	H Nb	161	50.0	1.50	2,790	1,960
111	22	H Nb	180	57.7	1.73	2,972	1,715

TABLE I—*contd*

Number	Age	Race	Height, cm	Weight, kilos	Body surface, sq m	Vital capacity, c c	Vital capacity per sq m, c c
34	22	A I	182	52.2	1.68	4,030	2,400
44	22	H Nb	170	56.3	1.64	2,724	1,660
51	22	H Nb	168	54.1	1.61	2,298	1,428
62	22	H Br	163	55.5	1.60	2,626	1,642
71	22	Moh	175	54.1	1.66	2,412	1,452
72	22	H Nb	159	45.0	1.43	2,854	1,995
75	22	H. Br	170	53.7	1.62	2,840	1,752
77	22	H B ₁	160	45.0	1.44	2,578	1,788
80	22	H Nb	165	48.2	1.51	2,956	1,956
90	22	I C	169	60.0	1.69	3,578	2,112
98	22	H Br	168	44.2	1.47	2,956	2,010
102	22	I C	156	43.3	1.39	2,510	1,805
2	23	I C	170	50.7	1.58	3,150	1,993
21	23	S C	164	47.0	1.49	2,296	1,510
14	23	H Br	160	62.0	1.65	2,624	1,595
36	23	A I	173	61.0	1.73	3,442	1,990
96	23	H Nb	163	50.8	1.53	2,956	1,928
67	23	H Nb	160	52.0	1.53	3,440	2,224
54	23	H Nb	172	65.0	1.78	3,790	2,130
76	23	H Nb	161	46.8	1.47	2,590	1,764
89	23	H Nb	170	50.9	1.55	2,790	1,765
6	23	Moh	165	57.2	1.62	2,938	1,810
68	23	H Br	163	63.2	1.68	3,034	1,802
99	23	H B ₁	176	61.4	1.75	3,576	2,040
95	23	H Br	174	80.9	1.96	3,280	1,672
104	23	H Br	174	46.4	1.54	2,956	1,916

TABLE I—*concl'd*

Number	Age	Race	Height, cm	Weight, kilos	Body surface, sq m	Vital capacity, c c	Vital capacity per sq m, c c
35	23	A I	165	52.7	1.57	3,360	2,140
41	23	H Nb.	163	52.7	1.56	2,708	1,736
59	23	I C	166	54.5	1.61	3,020	1,878
82	23	H Nb	164	59.1	1.64	2,956	1,800
92	23	I C	169	60.0	1.69	3,118	1,840
97	23	H Br	168	53.2	1.60	2,494	1,560
101	23	H Br	164	52.7	1.57	2,460	1,572
106	23	H Nb	155	55.8	1.54	2,378	1,542
110	23	H Br	165	50.8	1.55	2,888	1,862
13	24	H Nb	169	51.5	1.59	2,460	1,546
40	24	S C	161	52.0	1.53	2,624	1,720
4	24	S C	166	60.2	1.68	2,460	1,464
45	24	H Nb	159	46.4	1.44	2,790	1,940
5	24	H Nb.	163	51.0	1.51	3,118	2,030
12	24	H Br	163	66.0	1.68	2,870	1,708
27	24	I C.	164	64.1	1.70	3,886	2,284
70	25	S C	157	50.0	1.48	2,510	1,694
63	25	Moh	155	56.8	1.48	2,460	1,662
84	25	H Nb	173	61.8	1.74	3,740	2,148
93	25	H Nb	166	55.8	1.62	2,526	1,566
24	29	H Nb	167	56.3	1.63	2,362	1,450

TABLE II

Number	Age	Sitting height cm	Chest circum- ference, cm	Chest expansion, cm	Vital capacity, c c
108	18	89 0	86 1	4 6	3,248
109	18	75 5	74 6	3 1	2,000
48	18	88 4	82 5	2 6	2,660
9	19	91 4	75 2	3 3	3,156
43	19	82 5	83 5	4 6	2,658
4	19	85 1	86 2	4 7	3,642
19	19	88 4	82 6	2 5	2,790
20	19	90 7	76 0	3 0	2,772
42	19	88 4	78 8	2 5	2,460
1	20	87 6			3,690
38	20	84 0	84 7	2 5	2,460
65	20	90 4	84 5	6 5	2,956
28	20	85 6	84 8	3 0	3,442
29	20	86 4	82 3	4 5	3,610
37	20	81 3	80 7	6 3	3,200
47	20	91 5	77 5	2 5	2,460
87	20	88 4	80 6	3 8	3,280
15	20	82 6	86 3	2 6	2,694
26	21	84 7			2,790
17	21	87 2	77 3	2 5	2,710
16	21	87 2	84 6	2 5	2,758
56	21	89 2	83 8	2 6	2,330
31	21	98 0	96 4	5 8	4,640
10	21	83 3	86 8	8 5	4,150
8	21	85 6	83 8	5 1	2,956
23	21	84 8	83 8	1 6	2,902

TABLE II—*contd*

Number	Age	Sitting height, cm	Chest circumference, cm	Chest expansion, cm	Vital capacity, c.c.
78	21	88.9	81.9	3.8	3,280
105	21	83.8	80.0	2.6	2,872
100	21	82.3	76.3	2.5	2,460
32	21	87.5	90.8	3.8	4,130
55	22	86.6	79.4	3.9	2,460
39	22	87.7	84.6	2.5	2,166
85	22	83.8	83.3	2.6	3,524
73	22	84.5	88.7	5.7	2,956
38	22	85.6	84.3	3.5	2,460
11	22	84.7	83.5	3.0	3,082
61	22	84.3	82.6	2.5	3,138
112	22	94.0	85.7	3.7	3,200
83	22	82.0	82.9	3.2	2,790
111	22	90.1	86.3	2.6	2,972
2	23	85.1			3,150
21	23	87.0			2,296
14	23	87.2			2,624
36	23	93.0	89.6	3.9	3,442
96	23	83.5	81.9	3.8	2,956
67	23	86.8	81.5	3.0	3,440
54	23	88.1	96.9	4.2	3,790
76	23	83.8			2,590
89	23	84.2	82.0	2.6	2,790
6	23	81.3	98.3	4.0	2,938
68	23	87.0	92.9	2.9	3,034

TABLE II--concl'd

Number	Age	Sitting height, cm	Chest circumference, cm	Chest expansion, cm	Vital capacity, c c
99	23	85 0	92 7	2 6	3,576
95	23	91 7	98 1	4 2	3,280
104	23	84 9	75 6	3 8	2,956
13	24	83 8			2,460
40	24	83 5	87 1	3 9	2,624
4	24	85 1	92 2	3 8	2,460
45	24	83 8	82 3	4 5	2,790
70	25	81 3	83 2	3 7	2,510

TABLE III

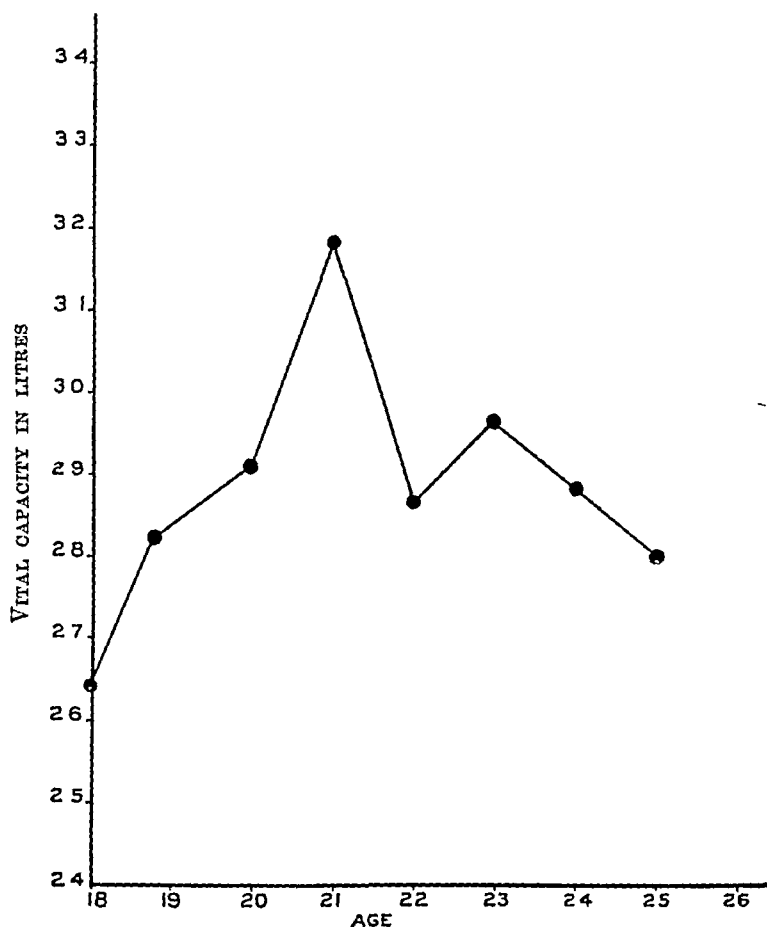
Average vital capacity according to age

Age	Number of subjects examined	Average vital capacity, c c	Average vital capacity per sq m, c c
18	4	2,642	1,687
19	8	2,821	1,827
20	18	2,913	1,848
21	16	3,182	2,006
22	22	2,869	1,836
23	23	2,967	1,830
24	7	2,887	1,812
25	4	2,809	1,768
29	1	2,362	1,450
Total Average	103	2,929	1,849

Standing height—As early as 1846, Hutchinson decided that, of all measurements, height bears the closest relationship to vital capacity. West (1920) found

FIG 1

Showing relation of vital capacity to age



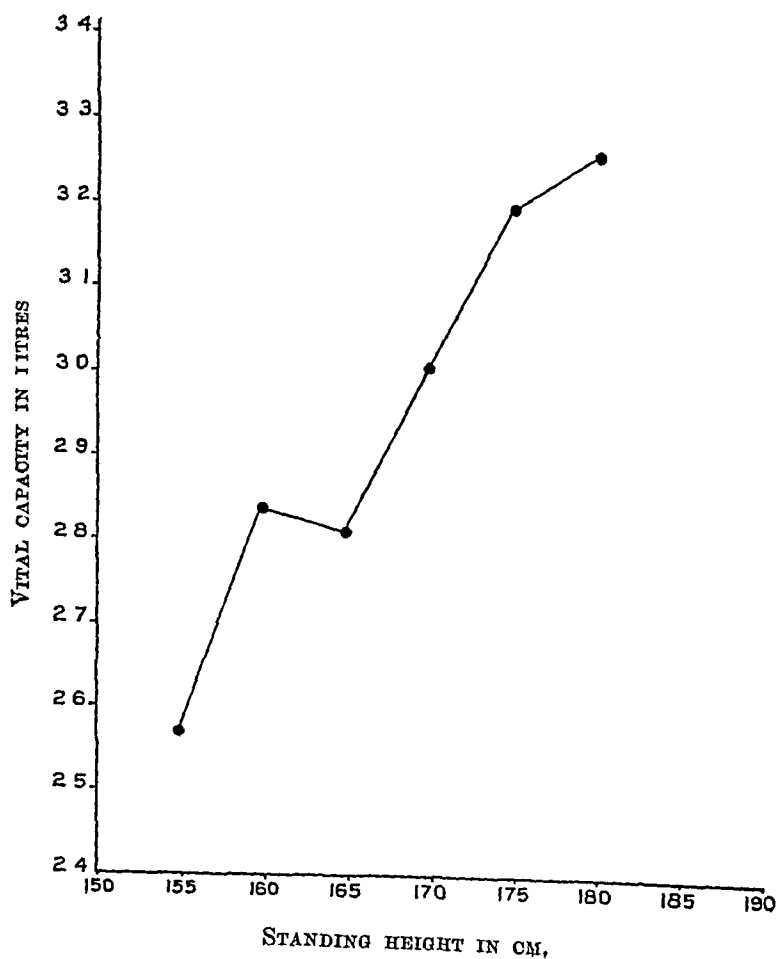
the height standard very useful in quick clinical work and calculated that every centimetre in height corresponded to 25 c c of air in men and 20 c c in women. Edwards and Wilson found in children the correlation between the vital capacity and height more intimate than between the vital capacity and body surface or weight. Each centimetre corresponded to 15.5 c c. In Table IV the correlation between the vital capacities of the 103 subjects and their standing heights in centimetres is shown (see Fig 2). A definite relationship between the two is found to exist. Omitting the first and the last items showing only one subject for the height noted, the average for each centimetre works out to be 17.5 c c in men.

TABLE IV

Average vital capacity according to height

Number of subjects examined	Height, cm	Average vital capacity, c c
1	150	2,000
6	155	2,565
16	160	2,838
38	165	2,810
28	170	3,003
10	175	3,191
3	180	3,258
1	185	4,640

FIG 2

Showing relation of vital capacity to standing height

Weight—Dreyer (1919) presented a formula based on body weight only, but after further study came to the conclusion that the vital capacity is more closely correlated to the body surface. In Table V the vital capacities according to weights in kilograms are shown. It is found that there is no close correlation between the vital capacities and the weights. Increase in weight is often due to obesity of varying degree and according to Myers obesity decreases the vital capacity because of the deposit of fat in the abdomen and the thorax. But I find that when the increase in weight is due to good physique and muscular development, the vital capacity does have a relationship with the weight as seen by comparing the subjects in Table VI with good physique with the subjects in Table VII with obesity of varying degree.

TABLE V
Average vital capacity according to weight

Number of subjects examined	Weight, kilos	Average vital capacity, c c
1	35	2,000
1	40	2,542
19	45	2,768
28	50	3,290
35	55	2,850
11	60	3,168
4	65	3,395
2	70	4,385
1	80	3,280

TABLE VI.

Number	Subject number	Race	Age	Weight, kg	Vital capacity, c c
1	31	A I	21	72.0	4,640
2	32	H Br	21	69.5	4,130
3	54	II N Br	23	65.0	3,790
4	27	I C	24	64.1	3,886
5	84	II N Br	25	61.8	3,740

 Date -

1	1
2	1
3	1
4	1

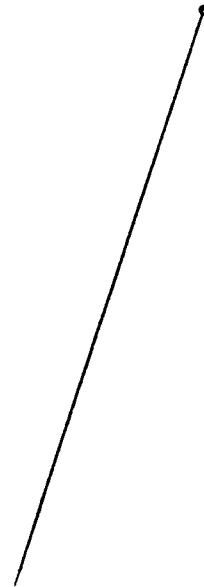
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Body surface — P_1

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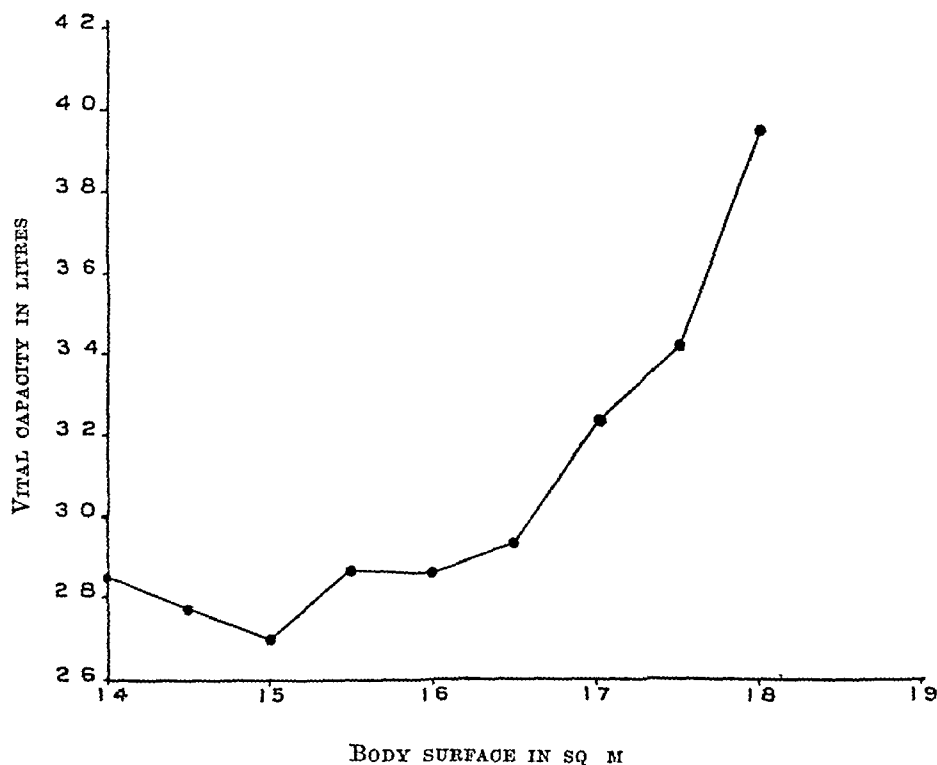


 6 98 100

body surface is found to be 1.85 litres. The average for areas ranging from 1.40 square metre to 1.60 square metre is practically on a level but shows a rise from 1.65 square metre upwards (see Fig. 3). There is a correlation between the vital capacity and the body surface in as much as there is a fall of vital capacity below 1.40 square metre and a rise above 1.60 square metre.

FIG. 3

Showing relation of vital capacity to body surface



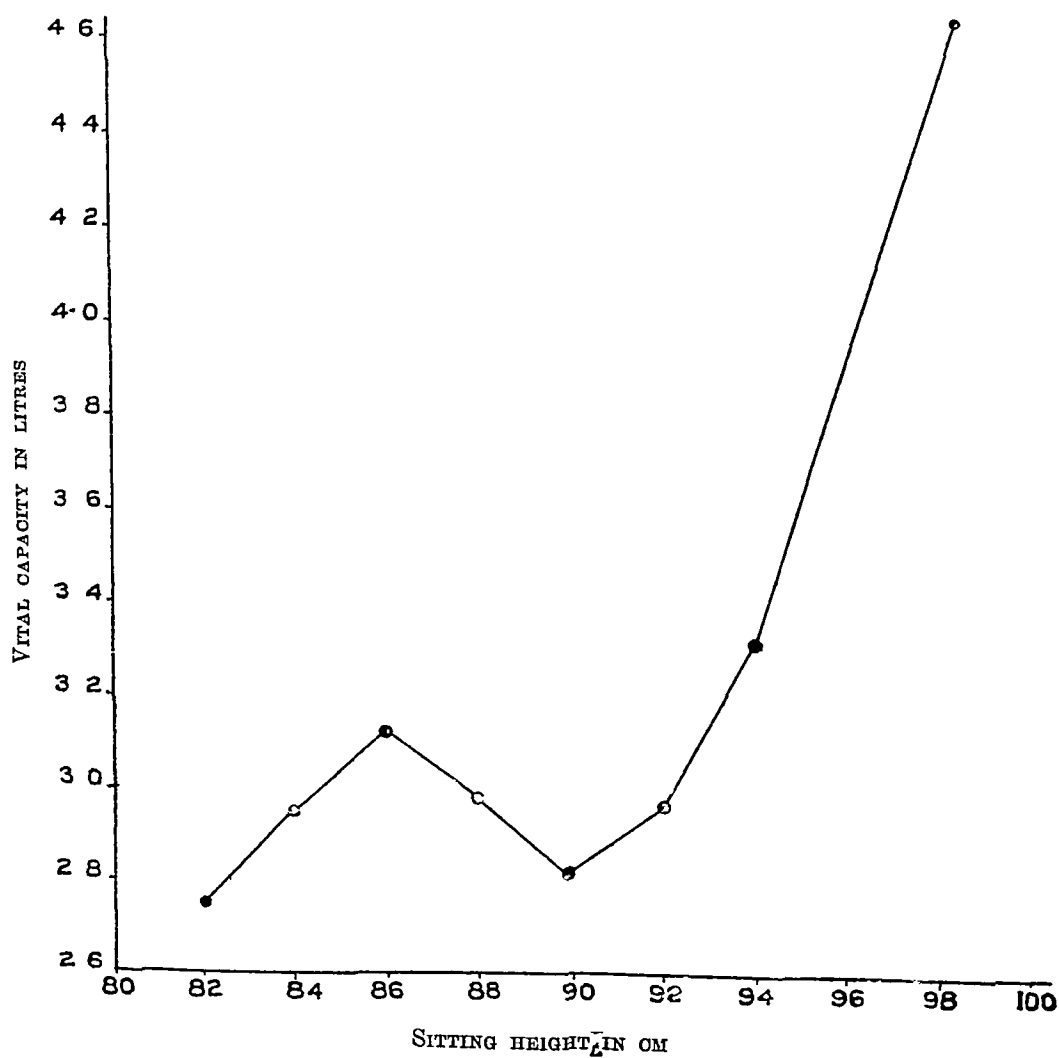
Sitting height—Dreyer (1919) thought that this measurement was valuable. Myers states that other workers have pointed out since Dreyer's publication that the sitting height standard shows more variation than other standards and is not materially better than the standing height standards. Table IX represents the average vital capacity according to sitting height. The sitting heights were measured according to the specific directions given by Dreyer. The measurement gives the distance between the ischial tuberosities and the top of the head. In the Table it will be seen that the vital capacity is more or less at one level between the heights ranging from 82 to 92 cm. and shows a marked rise above that height (see Fig. 4). So this measurement does not seem to be of much value.

TABLE IX

Number of subjects examined	Sitting height, cm	Average vital capacity, c c
7	82	2,750
16	84	2,941
10	86	3,120
14	88	2,978
5	90	2,820
3	92	2,965
2	94	3,321
1	98	4,640

FIG 4

Showing relation of vital capacity to sitting height



Chest circumference—This measurement again was found by various workers from Hutchinson downwards to show more variation than some of the other standards, though others like Arnold, Dreyer, etc., employed it in their studies. Table X represents the average vital capacity according to average chest circumference. The measurement was taken at the level of the nipples and the average of the circumferences on forced inspiration and forced expiration was noted. No correlation is found to exist between the chest circumference and the vital capacity. But taking the chest expansion alone there is found to be a definite relationship (see Table XI and Fig. 5).

TABLE X.

Number of subjects examined	Average chest measurement, cm	Average vital capacity, c c
1	74	2,000
4	76	2,836
3	78	2,553
4	80	2,953
10	82	3,024
13	84	2,786
6	86	3,318
3	88	2,839
2	90	3,786
3	92	3,023
<i>Nil</i>	94	
2	96	4,215
1	98	3,280

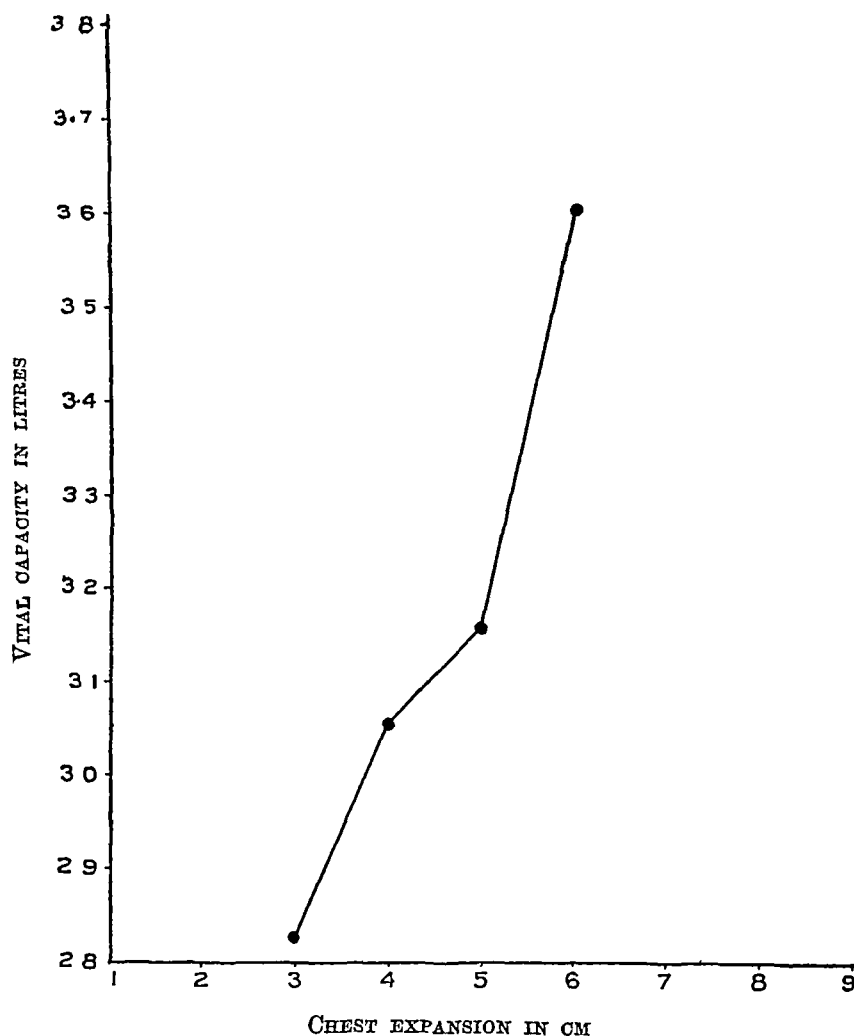
TABLE XI.

Number of subjects examined	Chest expansion, cm	Vital capacity, c c
1	2	2,920
25	3	2,822
15	4	3,051
6	5	3,151
3	6	3,599
1	7	2,956
1	9	4,150

It will be seen from the foregoing description of the correlations between the vital capacity and other measurements, that variation in vital capacity does not depend entirely on any one particular measurement and no standard based on any one measurement can be relied on. For finding the normal vital capacity,

FIG 5

Showing relation of vital capacity to chest expansion



the standards based on more than one factor, i.e., age, body surface, standing height and chest expansion, should be considered. Even then, as Myers observes, the vital capacity test is not infallible and individual variation offers one of its greatest limitations.

It has been said that race and nationality definitely affect vital capacity. In the cold climate of the Western countries, owing to the high rate of metabolism and increased oxygen consumption, there is constant increased pulmonary ventilation. In those countries, there is also a tendency for constant physical exercise, resulting in constant increased depth of the inspiratory excursions. For these reasons, the chests are bound to be adapted for larger vital capacities. In the warmer climates, just the reverse conditions prevail and so the normal standards are bound to be low. But in all cases (Table XII) where there was good physique and good chest expansion as a result of physical training, the vital capacity was found to be high, i.e., equal to or even a little more than the Western standard.

TABLE XII

Subject number	Race	Age	Height, cm	Weight, kg	Body surface, sq m	Chest expansion, cm	Vital capacity, c.c.	Vital capacity per sq m, litres
10	S C	21	164	54.0	1.58	8.5	4,150	2.65
31	A I	21	185	72.0	1.95	5.8	4,640	2.38
32	H Br	21	171	69.5	1.81	3.8	4,130	2.28
54	H N Br	23	172	65.0	1.78	4.2	3,790	2.13
1	H N Br	20	170	56.2	1.64		3,690	2.25
29	A I	20	177	52.7	1.64	4.5	3,610	2.20

So, in my opinion, the lower standards of vital capacity generally obtainable in tropical countries like India are due not to race or nationality but to climate, less tendency for exercise, low metabolism and poor chest expansion.

SUMMARY

With a view to find out the standards of vital capacity in South India, the vital capacities of 103 male medical students of 18 to 29 years of age from the different parts of the Madras Presidency were estimated, and certain measurements of the body, such as standing height, weight, body surface, sitting height and chest circumference, were recorded.

The correlation between the vital capacity and the various measurements is discussed. It is found that the maximum is reached at the 21st year. A definite correlation is found to exist between the vital capacity and the standing height and the chest expansion. With regard to body surface the vital capacity is on a

level for surface area from 1.4 to 1.6 square metre and shows a decline below 1.4 square metre and a rise above 1.6 square metre. The relationship is not definite with regard to weight, sitting height and chest circumference.

The average vital capacity for all the subjects examined is found to be 2.93 litres, 1.85 litres per square metre and 17.5 c.c. per centimetre of standing height. It is considered that for finding the normal vital capacity, the standards based on age, body surface, standing height and chest expansion should be taken note of. Among the subjects examined, there were a few cases with good physique and good chest expansion having vital capacities more or less equal to the Western standards.

The opinion is put forward that the low vital capacity, generally obtainable in South India, is due not to race or nationality but to the warm climate, less tendency for exercise, low metabolism and poor chest expansion.

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EXPERIMENTS ON PROTECTION OF MONKEYS WITH ANTI-VACCINIAL SERUM AGAINST THE VIRUSES OF VACCINIA AND VARIOLA

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[Received for publication, January 20, 1932]

INTRODUCTION

It has been noted that an anti-vaccinia serum suitably prepared and administered in suitable doses either intravenously or subcutaneously to experimental animals confers some degree of protection against a subsequent inoculation of vaccinia virus. On account of the close affinity between vaccinia and variola viruses, it has been suggested that such a serum could be used with advantage in the prophylaxis and treatment of smallpox. One limitation is, however, that such a protection is not demonstrable once the vaccinia virus is firmly established in the tissues. Andrews (1929), who studied the time-factor in such experiments, noted that such a protection could only be observed when the serum was at least five minutes ahead of the virus. With simultaneous inoculations of both the virus and the anti-serum, the development of the local lesion was practically unaffected. Thompson (1930) noted that an animal receiving 20 c.c. of potent serum intravenously gave no reactions to undiluted virus injected intradermally 24 hours later. Such complete protection was not, however, obtained when smaller amounts of the immune serum were used. Craigie and Tulloch (1931) confirmed the observations of Andrews and found that by injecting rabbits intravenously with a flocculating

serum in doses of 2 c c per kilo of body-weight, and vaccinating them one hour later with vaccinia virus, a considerable degree of passive immunity could be conferred in them

As Craigie and Tulloch have pointed out 'this method of testing the degree of protection is probably very artificial, in that the virus is introduced into the skin in a highly concentrated form and its introduction is accompanied by severe trauma' The virus therefore gets very rapidly established in the tissues or, in other words, the incubation period in experimental vaccinia in rabbits is of a short duration Perhaps it is due to this factor, that when the virus is ahead of the immune serum even by a few minutes, the protective action of the latter is not readily demonstrated The question is obviously of some importance for to be of any use, the serum must be shown to have some neutralizing effect even when the infection is so firmly established As suggested by the Director of the Institute (Lieut -Colonel H H King, I M S), it was therefore decided to repeat the foregoing experiments using monkeys as test animals, as the course of vaccinia in them is of a much longer duration than it is in rabbits, and later, if successful, to try the effect of anti-serum in the treatment of smallpox The experiments were done both with the viruses of vaccinia and variola as monkeys are known to be susceptible also to the latter

TECHNIQUE AND MATERIALS

The anti-vaccinal serum used in these experiments was obtained from buffaloes These animals are at present employed as a routine for the maintenance of potency of our seed lymph (Nijland's method See *Annual Report of the King Institute*, 1923) A buffalo was vaccinated in the abdomen with lymph which had previously passed through another buffalo The lymph was removed after 120 hours and the lesions allowed to heal Ten days later the same buffalo received an intravenous injection of 10 c c of 1 in 100 dilution of the buffalo lymph Two more injections were given at 7 days interval, and 10 days after the last injection the animal was bled through the jugular vein The blood was left in the cold room at 27°F No preservative was used It was found that a 1 in 40 dilution of the serum completely inactivated an equal volume of a 1 in 300 dilution of vaccine lymph having the potency noted below while its flocculating titre was 1 in 40

For vaccinia virus the routine calf lymph manufactured in the Institute was employed One cup was reserved for these experiments It was potent as tested on a calf in a dilution of 1 in 20,000 The serum was titrated against this virus

Protective experiments with this serum were carried out in monkeys—*Macacus sinicus*—using both the viruses of vaccinia and variola The animals were vaccinated by scarification with 0.1 c c of either diluted lymph (usually

1 in 500) or with variolar lymph obtained from cases of smallpox and immediately afterwards a suitable dose of immune serum was administered subcutaneously

EXPERIMENTS WITH VACCINIA VIRUS

The course of vaccinia in monkeys is of a very much longer duration than what is usually seen in rabbits. No visible reaction is noticed for the first two days, papules appear on the 3rd day, vesiculation commences on the 5th, vesicles maturing some time during the 6th to 8th day. The scab falls off on or about the 14th day. The object of the experiment was to determine, if and to what extent, the course of vaccinia was affected by the subcutaneous administration of anti-vaccinal serum.

Experiment I

Two monkeys each weighing approximately 1,700 grammes were chosen. They were vaccinated by scarification on the skin at the back with 0.1 c.c. of the following dilutions of vaccine lymph, viz., 1 in 50, 1 in 100, 1 in 200 and 1 in 500. One of the monkeys received 10 c.c. of the anti-vaccinal serum subcutaneously and the other was left as control.

Result—All the dilutions took well in the control monkey, confluent vesiculation appearing with the 1 in 50 dilution and discrete vesiculation with the remaining three dilutions of vaccine lymph. In the other monkey that had received the serum, discrete vesiculation appeared with 1 in 50, 1 in 100 and 1 in 200 dilutions. The protective action of anti-vaccinal serum was thus evident against 1 in 50 dilution of lymph and particularly against 1 in 500 dilution which failed to take entirely (see Plate LXI, figs 1 and 2).

Experiment II

The above experiment was repeated, but only 1 in 500 dilution of vaccine lymph was used in both the monkeys. The dose of immune serum was 10 c.c. as before.

Result—Normal vesiculation was obtained in the control monkey but there was no 'take' at all in the other monkey. The result in the first experiment, viz., that 10 c.c. of immune serum gave full protection against 0.1 c.c. of 1 in 500 dilution of vaccine lymph, was thus confirmed (see Plate LXI, figs 3 and 4).

Experiment III

As a control the same experiment was repeated, using normal buffalo serum instead of the immune serum. Normal takes appeared in both the monkeys. Normal buffalo serum had thus no protective action against vaccinia virus.

In the foregoing experiments, the serum and the virus were both introduced almost simultaneously. In the following experiments the serum was given at varying intervals after vaccination with vaccine lymph.

Experiment IV

Four monkeys of approximately the same weight were chosen. They were all vaccinated with 0.1 c.c. of 1 in 500 dilution of lymph as before. Number one was left as a control. Monkeys Nos. 2 and 3 received 15 c.c. of immune serum at 1 hour and 4 hours respectively after vaccination. Monkey No. 4 received 5 c.c. of immune serum immediately after vaccination and 10 c.c. the next day, i.e., 24 hours after vaccination.

Result—Monkeys Nos. 1 and 4 showed a normal take, reaction appearing on the 3rd day, i.e., at 72 hours. In monkeys Nos. 2 and 3 however, the vesiculation seemed retarded. There was only a few papules noticeable at 72 hours. At 96 hours a few more appeared while at 120 hours there was no appreciable difference in the 'takes' in all the four monkeys. Excepting for this late development of the reaction, the action of immune serum was not apparent in this series.

EXPERIMENTS WITH VARIOLA VIRUS

It would appear from the foregoing experiments that an anti-vaccinal serum administered as above, gives a definite degree of protection against a certain dose of potent vaccine lymph. Since the serological identity of vaccinia and variola viruses has now been definitely established (Craigie, 1930) it was decided to see if anti-vaccinal serum gave protection against the variola virus as well. Monkeys are particularly suitable for experiments of this nature. As was first pointed out by Copeman they react very readily indeed to variola virus when it is introduced into the skin by the usual skin scarification method. The susceptibility of monkeys to this virus had been tested in this laboratory on many occasions, and in no instance was a monkey found to be insusceptible to its inoculation. The development of the lesions may be described as follows—

The injury caused by scarification heals usually within 24 to 48 hours. For 72 hours, there are no signs of any commencing reaction. Copeman noted that on the 3rd day the inoculated site was covered with a very thin crust. When this is noted, a 'confluent take' finally appears. When a diluted virus is used (or material with a low virus content) only discrete papules appear on the 4th day. These seldom assume any degree of prominence till after the 6th day. Vesiculation appears on the 7th or 8th day. The vesicles get dried up and the resulting scabs do not fall off till during the 3rd week. Pitting of the skin at the site of old vesicles is usually seen. Generalization occasionally appears but was not met with in the present series.

Copeman found that in monkeys previous vaccination or variolation afforded protection against the subsequent inoculation of the corresponding virus. Later researches do not confirm this view, for although vaccinia protects against variola the action is not reciprocal to the same degree. It must be noted that one passage through the monkey does not always result in the transformation of the variola virus into vaccinia virus. Frequent passages through a series of animals are occasionally required, and it is only when such adoption occurs that passage animals show any immunity towards subsequent vaccination with vaccine lymph.

The general technique employed was the same as before. Variola virus was obtained for a case of smallpox in an unvaccinated child aged 2 months. Variola lymph was removed on the 6th day of disease. It was collected in 50 per cent glycerine water (one part of lymph and 14 parts of glycerine water, dilution of original material 1 in 15).

The diagnosis of smallpox in this case was never in doubt. Further from this variolar virus vaccine lymph was obtained ultimately by adopting the usual monkey and calf passages. The details are given later.

With this smallpox virus the following experiments were done —

Experiment V

Two monkeys each weighing about 1,700 grammes were taken and on the shaved skin of the back 0.1 c.c. of the virus suspension was introduced by scarification over an area 1 inch square. One of the monkeys then received 15 c.c. of anti-vaccinal serum while the other was left as a control.

Result — In the control monkeys no appreciable reaction was noted till the 4th day. On the 5th a few papules began to appear. The subsequent development was very rapid, the whole scarified area being covered over with a confluent eruption giving the appearance of a raised patch of vesiculated surface. The patch was then scraped lightly on the 8th day, the material was collected in 50 per cent glycerine water and preserved in the cold room at 27°F for further passages on animals with a view to cultivate it into vaccinia virus. The lesion ultimately healed and scabs fell off on the 17th day after inoculation. Distinct pitting was noted on the inoculated site.

In the other monkey which had received a dose of serum the reaction was considerably delayed. On the 7th day a few papules about 20 in number were noted on the scarified area. These developed into vesicles the next day. These were scraped and the material treated in the same way as before. The lesions healed rapidly and scabs fell off on or about the 12th day. No pitting was observed and hair had already grown on the part on the 17th day, i.e., when the scabs in the control monkey had fallen off (see Plate LXII, figs 5 and 6).

Conclusion —As compared with the reaction in the control monkey, some evidence of the protective action of the serum was obtained. But as the result was not entirely conclusive the experiment was repeated with a slight modification.

Experiment VI

Two monkeys were inoculated as before, but the virus was further diluted with equal parts of glycerine water (dilution 1 in 30). In the control monkey 0.1 c c of both the dilutions were introduced by scarification as before. The other monkey received 0.1 c c of 1 in 30 dilution of variolar lymph and in addition 15 c c of anti-vaccinial serum.

Result —The control monkey showed 9 vesicles with each of the two dilutions inoculated. The general development was the same as before. In the other monkey only one vesicle was seen (*see* Plate LXII, figs 7 and 8).

Conclusion —The two foregoing experiments show definitely that anti-vaccinial serum afford some protection against variola virus as well, when the serum is given simultaneously with the virus.

CULTIVATION OF VACCINIA VIRUS FROM THE MATERIAL OBTAINED FROM MONKEYS IN EXPERIMENT No I

As has been noted before the material from these monkeys was preserved in 50 per cent glycerine water in the cold room (27°F). A calf was subsequently vaccinated separately with material obtained from both the monkeys. However only with the material obtained from the control monkey about 3 vesicles were noted on the scarified area on the calf. These were scraped and the material passed on to another calf, when a reaction identical with that of vaccinia virus was obtained. It was thus confirmed that the case from which the material was obtained was of true smallpox.

The effect of giving the serum at varying intervals was then studied. For this purpose the variola virus was obtained from another case of smallpox from pustules on the 6th day of disease and it was collected in 50 per cent glycerine water in a concentration of 1 in 20. As before, from this variolar lymph, typical vaccinia virus was cultivated by the usual monkey and calf passages.

Experiment VII

Five young monkeys of approximately equal weight (1,200 grammes) were chosen. Each of them received 0.1 c c of variolar lymph which was introduced by skin scarification as before.

No 1 was left as control, No 2 received 15 c c of immune serum simultaneously with scarification with the virus, and monkeys Nos 3, 4 and 5 received 15 c c of

immune serum 1 hour, 4 hours and 24 hours respectively after scarification The results were as follows —

The control monkey — Definite evidence of confluent take at 72 hours The scarified area was raised and œdematous Normal course of development thereafter

Monkey No 2 — No reaction at all till the 8th day and it looked a failure On the 12th day 5 very small papules were noted They did not develop further

Monkey No 3 — Same as above Five papules were observed only after the 8th day

Monkey No 4 — A few vesicles were noted on the 5th day Few appeared later and on the 10th day about 24 vesicles in all developed

Monkey No 5 — General course of development the same as in monkey No 4, but on the 10th day about 45 vesicles were developed The reaction on that day looked more or less the same as was noted in the control monkey

It thus appears that unlike what happens with vaccinia virus, the serum gave definite protection against variola virus even when it was administered 4 hours after scarification When it was so given 24 hours after scarification, the reaction was considerably modified

The experiments were repeated on similar lines again and the results were generally confirmed These results are important, in that they show that an anti-vaccinial serum is likely to have some beneficial action in the treatment of smallpox, if it could be given sufficiently early in the disease, and so would justify the trial of anti-vaccinial serum for the treatment of smallpox in man

OBSERVATIONS WITH HUMAN IMMUNE SERUM OBTAINED FROM CONVALESCENT CASES OF SMALLPOX

From the foregoing experiments it is seen that a fairly large amount of immune serum was required to obtain an evidence of definite protection Probably with more prolonged immunization, a better serum for this purpose could be obtained For comparison therefore similar experiments were done both with the stock buffalo serum, and serum obtained from confluent cases of smallpox obtained during convalescence, i.e., after the scabs had fallen off completely Experiments were done both with the virus of vaccinia as well as of variola The technique was the same as used in the foregoing experiments However as the supply of convalescent serum was limited, both the sera were given in 10 c.c. amounts In monkeys receiving the convalescent serum no reactions appeared either with the virus of vaccinia or variola In monkeys receiving the buffalo immune serum a modified take appeared with both the viruses It was thus found that in two experiments so made, the results, obtained with convalescent serum, were far superior to those obtained with buffalo immune serum For experimental purposes, therefore, it is proposed to use the human convalescent serum as standard, and to hyper-immunize

the buffaloes to such an extent at least that the anti-vaccinial titres of both would be about equal

SUMMARY

1 The protective action of anti-vaccinial serum obtained from hyper-immunized buffaloes was studied in experimental infection of monkeys with the viruses of vaccinia and variola. The viruses were introduced by scarification and the sera were given subcutaneously in all cases.

2 When the serum was administered along with the virus considerable degree of protection was obtained both against the viruses of vaccinia and of variola.

3 When the serum was given at varying intervals, after vaccination with vaccine lymph, no absolute protection was demonstrated in any case but the course of vaccination was modified to a slight extent. On the other hand the serum gave considerable protection against variola virus even though it was administered 4 hours after the virus was introduced by scarification. Even if the serum was given 24 hours after, the course of the reaction was considerably modified.

4 Serum obtained from convalescent cases of smallpox showed a much higher degree of protection than did that obtained with the buffalo immune serum.

5 The results warrant an extension of efforts both to produce from animals an anti-vaccinial serum of high potency, and to investigate the benefit that may be obtained by the use of convalescent human serum.

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PLATE LXI

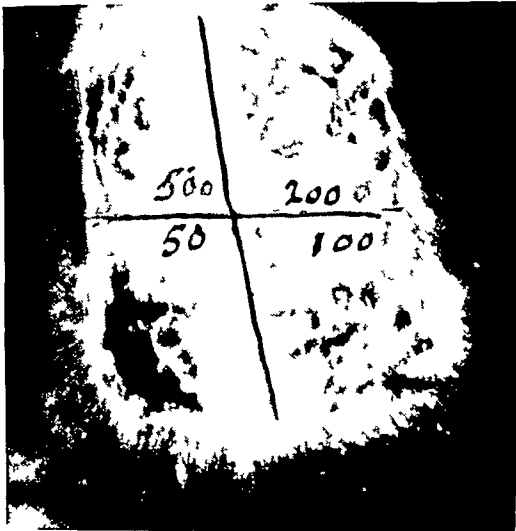


Fig 1—Control figures give lymph duutions

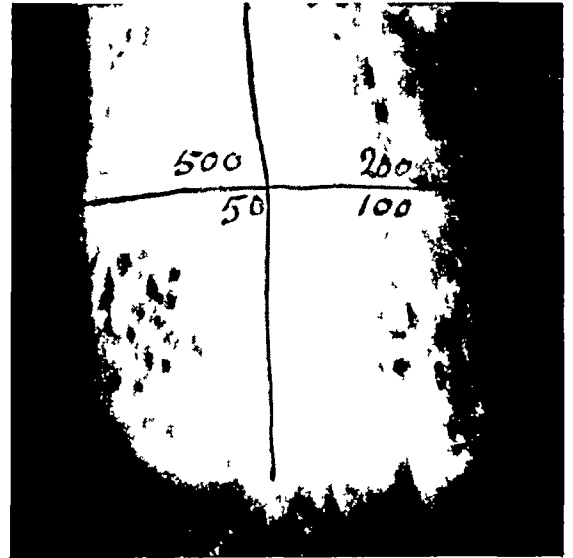


Fig 2—Experimental

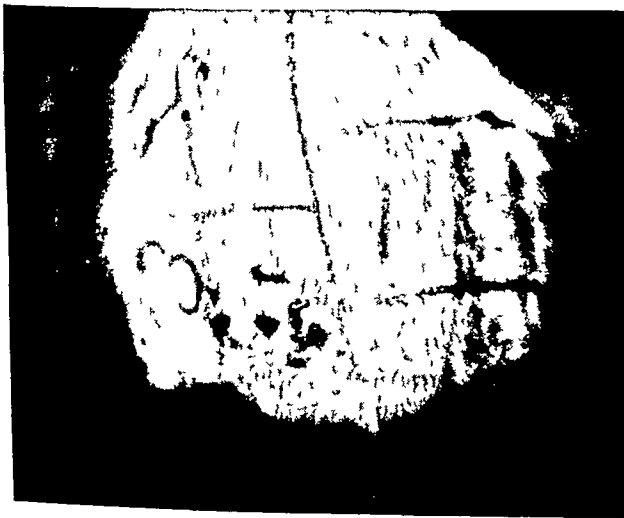


Fig. 3.

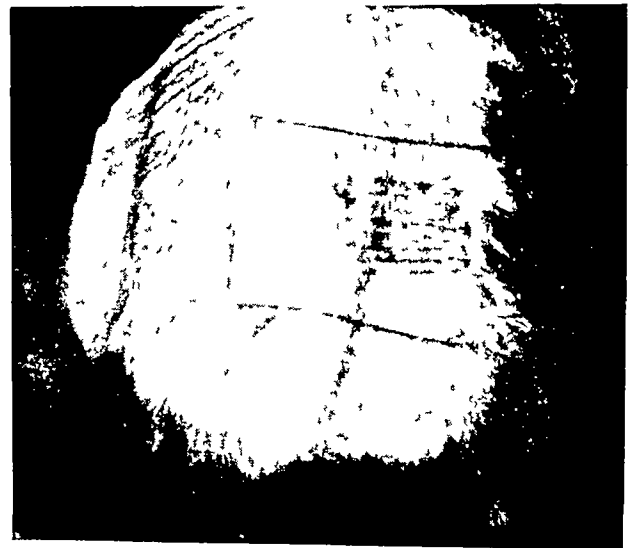


Fig. 4

PLATE LXII



Fig 5—Control



Fig 6—With anti-serum

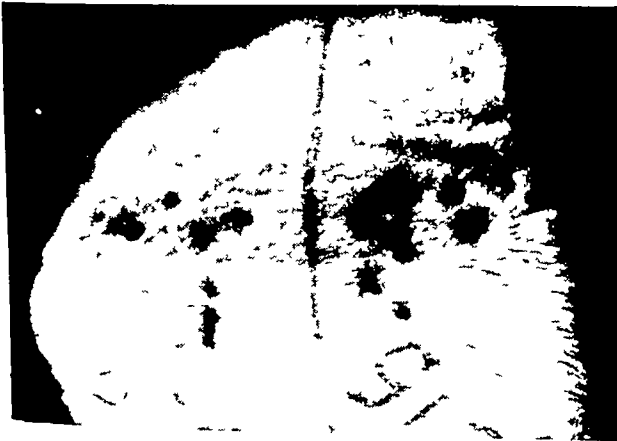


Fig 7—Control



Fig 8—With anti serum, the circle shows the extent of the swelling around the vesicle

PHARMACOLOGICAL ACTION OF BERBERINE

BY

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Indigenous Drugs Series No 32

[Received for publication, January 22, 1932]

THE alkaloid *Berberine* is well known in medicine. It occurs chiefly in *Berberis aristata* and other members of the berberis family (N O *Berberideæ*). It has also been found to occur in the roots and rhizomes of *Hydrastis canadensis* N O *Ranunculaceæ*. It is also present in a large number of plants belonging to the natural orders, *Menispermaceæ*, *Papaveraceæ* and *Rutaceæ*, etc., which have been employed in the indigenous medicine in India for many centuries. In both the Hindu and the Mohammedan medicine these plants have been used as diaphoretics and stomachics and also in the treatment of many skin diseases. The plant barberry is no less interesting in biology for long before Sir J C Bose started his experiments on the motor mechanism of plants, certain movements of the anthers of this plant had been recorded in response to external stimuli. Although berberine containing plants are largely used in the indigenous medicine in this country, the pharmacological action of berberine was not fully worked out. Interest has also been recently aroused in this drug on account of its successful use in the treatment of dermal leishmaniasis (oriental sore).

BERBERINE BEARING PLANTS

The alkaloid berberine occurs in a large number of plants of the barberry family, growing in the north and western parts of the Himalayas at an altitude

from 1,000 to 4,000 feet above the sea-level They grow in Bhutan and in the Nilgiris in the south of India, in the European and American forests they are also to be found Berberine is present to a greater or lesser extent in the following plants growing in India —

1 *Berberis aristata*, N O *Berberideæ*, commonly known as 'ophthalmic barberry' or 'Indian barberry' It is known as *chitra chotra* and *kashmal* in Hindi, *simlu*, *chitra* in Punjabi, *chitra* in Nepal and *chitra* in Persian It grows in the temperate Himalayas at an altitude of 6,000 to 8,000 feet

2 *Berberis asiatica* is known in the vernacular as *kilmora mate-hissi* or *chitra* It grows in the dry valleys of the Himalayas at an altitude of 3,000 to 7,500 feet, as well as on the Paresnath Hills in Bihar

3 *B. coriacea*, known in the vernacular as *kashmal* It is a large erect thorny shrub growing in the North-West Himalayas above 8,000 feet

4 *B. lycium*, known in the vernacular as *kashmal*, *chitra kushmul* and *darhalad* It grows in the dry and hot parts of the Western Himalayas at an altitude of 3,000 to 9,000 feet from Garhwal to Hazara

5 *B. nepalensis*, known in the vernacular as *aumdana*, *chior* and *mlkissi* It grows commonly on the outer Himalayas from the Ravi eastward to Khasia and the Naga Hills and also in the Nilgiris at an altitude of 5,000 feet

6 *B. vulgaris*, known in English as the 'true barberry' In the vernacular it is known as *zimshk*, *kashmal*, *chachar* It is a deciduous thorny shrub growing in the Himalayas from Nepal westwards, in shady forests at an altitude over 8,000 feet above the sea-level

A crude extract made from the berberine plants is largely used in the indigenous medicine and is sold in the bazar under the name of rasaut, rasanjana or rasavanti There is some difference of opinion as to whether *rasaut* should be regarded as a special preparation from the root of *B. lycium* only, or from *B. asiatica* or the two together Most of the extract met with on the market is derived from both the plants It is prescribed in doses of from 10 to 30 grains with butter in bleeding piles and as a bitter tonic and febrifuge Mixed with butter and alum *rasaut* is used as an external application for the eyelids in acute conjunctivitis With camphor and butter it forms a constituent of an ointment used against acne, pimples and indolent ulcers Surgeon Joseph Parker found it very useful in enlargement of liver and spleen

Besides the various species of berberis some other plants used in the indigenous medicine are said to contain berberine —

1 *Tinospora cordifolia*, N O *Menispermaceæ*, known in Sanskrit as *gudueh*, *pitlagur* (bile destroying), *bhishakprya* (dear to the physician) and *nirjara* (not perishing), and in Hindi and Bengali as *gillo* and *gulanha* It is a climbing shrub growing on a number of high growing trees in tropical India, Burma and Ceylon

2 *Coptis teeta*, N O *Ranunculaceæ* is known in Hindi as *Mamua*, *Mishmitila* and is reputed as an eye-salve. This plant is commonly known as 'gold thread' and is a native of the mountains bordering on Upper Assam. It contains as much as 8.5 per cent of berberine in the root.

3 *Thalictium foliosum*, N O *Ranunculaceæ*, is known in the vernacular as *pinjar* or *piljar*. The root resembles the liquorice root in appearance and is sold in the bazar under the name of *piljar* or *paranga*.

4 *Toddalia aculeata*, N O *Rutaceæ*, known in the vernacular as *Jangli-lal-mirch* or *Kandi* or *Dohan*. It is a climbing shrub found in the lower Himalayas, and Bhutan at an altitude of 5,000 feet above the sea-level.

5 *Coscinum fenestratum*, N O *Menispermaceæ*, known in Hindi and Bengali as *Dharhaldi*. It is a climbing plant which grows plentifully in the forests of Western India. The wood yields a dye resembling turmeric.

6 *Xanthoxylum alatum*, N O *Rutaceæ*, known in the vernacular as *Tegphal* or *Tamur*. This is a common shrub or bush growing in the temperate Himalayas, Bhutan and Khasia Hills. The bark of this plant and several other species of the same genus are said to contain berberine but we have not been able to detect any alkaloid in it.

7 *Jateorrhiza calumba*, N O *Menispermaceæ*, known as *Kalamkyn* in Hindi, *Kalamba* in Tamil and *Kalamkachi* in Bombay. It grows in the forests of East Africa along the Mozambique coast. The drug appears to have been first introduced into India by the Portuguese.

Later researches have, however, shown that *Tinospora cordifolia*, *Thalictium foliosum*, *Xanthoxylum alatum*, and *Jateorrhiza calumba* do not contain any berberine at all.

THE ALKALOID BERBERINE

Berberine $C_{23}H_{19}NO_5$ is the chief constituent of *Berberis aristata* and *Hydrastis canadensis* 'Golden seal' occurring to the extent of nearly 2.5 per cent. Berberine is an intensely yellow and bitter alkaloid. It is widely distributed in the root and bark and is the main source of the yellow colour of these plants. It crystallizes out of the solution with acetone and chloroform. It melts at 144°C and when acidulated with sulphuric acid in a test tube and brought in contact with chlorine water it gives a blood red ring at the junction. It precipitates with nearly all the alkaloidal precipitants such as Mayer's reagent, potassium iodide, chromic and picric acids, platonic acid, gold chloride, sulphuric acid and other acids in fairly low dilution and bromine water.

Berberine base dissolves in 4.5 parts of water at 21°C . A number of salts such as carbonate, sulphate, hydrochloride, etc., have been prepared. They all have a yellow colour and are very sparingly soluble in water, except the acetate and the phosphate which have a solubility of 1 in 15 parts of water. The solubility

of the sulphate is 1 in 150, but the acid sulphate is more soluble, the hydrochloride is soluble 1 in 400 parts of water. The solubility in water increases on warming the solution or on the addition of alcohol and benzol. A 2 per cent solution of berberine sulphate gives a thick heavy precipitate when it comes in contact with normal saline or blood. In our experiments we used a 1 per cent solution of the freshly prepared acid sulphate having a pH of 5.8.

Pharmacological action of berberine—Sollmann (1892) reported that about 85 per cent of the circulatory effects of hydrastis were due to the presence of berberine as its quantity was $1\frac{1}{2}$ times more than hydrastine and its action about 7 times as strong. The lowering of temperature, increase of intestinal movements and death of the animal after hydrastine, it was suggested, were really due to berberine. E. Merck (1911) gave a brief description of the action of berberine.

Action on bacteria and protozoa—Das Gupta and Dikshit (1929) found that in such dilutions as 1 in 80,000 berberine inhibits the growth of *Leishmania tropica* in a modified N N N medium. A comparison with protoplasmic poisons such as emetine, quinine, etc., will show that the action of this alkaloid on *L. tropica* is a specific one. This is obvious from the fact that whereas 1 in 1,000 of quinine will definitely inhibit the growth of *L. tropica* and in similar concentrations of emetine the leishmania will grow luxuriantly, berberine in 80 times that dilution stops the growth of this protozoa.

Berberine has little or no action on the growth of bacteria. Even in such concentrations as 1 in 200 to 500, intestinal bacteria grow very well and the growth of such organisms as streptococci and staphylococci was not inhibited.

Local action—Application of berberine to the intact skin does not produce any inflammatory reaction, the conjunctiva is not inflamed if a 1 per cent solution is applied to it. The corneal reflex is not altered after local application of the drug in rabbits. When injected hypodermically or intramuscularly in the cat and the rabbit, there is no evidence of any inflammation at the site of injection. Microscopical examination of the tissues shows much exudation of lymph between the muscle fibres. There is dilatation of the blood vessels but no migration of the leucocytes can be detected even 24 hours after the injection. The lymph is slowly absorbed and the injections as a rule are painless.

Action on the blood—Berberine does not alter the clotting time of the blood nor does it produce any marked changes in the blood corpuscles. A 10 per cent solution floated over the serum produces a white ring at the junction of the two fluids. If a few drops of a solution of berberine sulphate are added to a small quantity of Locke's fluid, a thick flocculent precipitate is formed within 15 minutes. The precipitate seen after addition of a berberine salt to the blood or serum is chiefly due to interaction of the alkaloid with inorganic salts of the blood though the proteins may also take some part in it.

Toxicity—Toxicity of the alkaloid was determined on frogs, rats and rabbits. The minimum lethal dose of berberine for frogs when given in the anterior lymph sac is 0.1 mg per gramme of body-weight. The most prominent symptoms after a toxic dose are marked slowing of the respiratory movements, the reflexes are diminished and there are signs of muscular weakness.

Rats are killed by a dose of 0.25 mg per gram when the injection is given through the tail vein though some may live for 48 hours. As a rule they die within 24 hours.

The minimum lethal dose for rabbits is 0.1 g per kilo when given subcutaneously. With such doses there is slowing of respiration at first but this usually passes off. When the alkaloid is given intravenously in such doses as 75 mg per kilo through the ear-vein there is a marked acceleration of the respiration followed by slowing, convulsions may occur before death.

In the dog and the cat under anaesthesia, the dose required to stop the respiration varies between 50 to 100 mg in animals weighing between 2 and 4 kilos. Death is usually due to respiratory paralysis although a very marked depression of the heart is also observed. Usually the heart continues to beat after the respiration stops, and if artificial respiration is given it may continue to beat for a long time after.

Post-mortem examination of rats and rabbits who were given minimum lethal doses of the alkaloid shows two prominent lesions. The lungs are in all cases very congested, become almost hæmorrhagic and solid. The auricles are markedly dilated, but this is not always the case with the ventricles, the left ventricle may even show contraction. Marked congestion of the lungs and dilatation of the auricles are very constant features in these animals. The liver is pale and yellowish in colour and does not show any signs of congestion, the spleen, the pancreas and the gut show no marked changes. The kidneys show no evidence of congestion or discoloration and this would appear to suggest that the alkaloid is not excreted by the kidneys to any large extent. Except the heart, the lungs and the liver, all the other organs of the body appear to be quite normal to the naked eye.

Absorption—The salts of the alkaloid are absorbed completely when given intramuscularly or hypodermically. When 5 to 10 c.c. of a 1 per cent solution are injected, there is generally no trace of the solution seen at the site of injection after 48 hours. Sometimes small granules are visible especially when the site is examined before 24 hours have elapsed. The drug is completely absorbed within 48 hours leaving no traces behind. The fact that in man the alkaloid is detected in the urine within about 20 to 30 minutes after its oral administration shows that the drug is absorbed from the gastro-intestinal tract fairly rapidly.

Digestive system—The movements of the stomach in unanaesthetized cats were studied by inserting a balloon in the stomach through a gastric fistula. The animal was operated 4 weeks before the records were taken and was trained to lie

down on the operation table quietly for hours together. The drug was either injected hypodermically or was introduced into the stomach by means of a fine catheter inserted through the opening in the stomach along with the rubber balloon. The immediate effect of the local application of any drug or saline is generally a temporary inhibition of the contractions of the stomach. The movements, however, reappear quickly and come to the original magnitude in a couple of minutes. Introduction of 3 c.c. of a 1 per cent berberine solution inhibits the movements immediately after the application of the drug, but after absorption the amplitude of contractions is greatly increased (Graph I, *a* and *b*). Similar results are obtained after subcutaneous injections, the stimulation of contractions, therefore, is not due to local irritation.

Movements of the intestines in animals without anaesthesia were taken either with a Thiry-Vella fistula or with the exteriorized loop of intestines. The Thiry-Vella fistula was made in the usual way, a tube of about 5 inches in length being left in the abdominal cavity for inserting a balloon and taking tracings.

Exteriorization of the intestines was done after the technique described by Barcroft and Stephens for the exteriorization of the spleen. A dumb-bell shaped incision was made in the median line in a dog and small loop of intestines about 3 to 4 inches in length was taken out of the wound. The wall of the intestinal loop was sutured to the two ends of the incision by catgut. The peritoneum with the vessels and nerves was lodged in the linear portion of the incision in a fan-like fashion. Two or three stitches were put to close up the linear incision, the needle passing through the skin, the subcutaneous tissue, the fascia and muscles on one side, pierced through the peritoneum without injuring the vessels or nerves and passed through the muscle, fascia, subcutaneous tissue and skin through the other side. The two ends of the suture were tightened up in such a way as to close the wound without interfering with the blood supply. Haemorrhage, if any, was controlled and the wound together with the loop was smeared with a thick layer of sterilized vaseline. The loop was protected with a small wooden box and the dog was muzzled. There was marked inflammatory reaction in the loop after 24 hours and the movements of the piece of gut were completely paralysed for 2 to 3 days. The inflammation gradually subsided and after a week the movements were clearly visible, and the loop was ready for experimental observations. The dressings consisted in washing the wound with warm lysol and covering again with sterilized vaseline. The tracings were taken by making a slit in the wall of the gut and inserting a small balloon inside the lumen. The drug was introduced into the lumen of the intestinal canal with a syringe, the needle piercing the gut wall. Such injections are painless and the animal shows no sign of discomfort while the injection is given.

Of the two methods, Thiry-Vella fistula was found to be more satisfactory but the advantage in the exteriorized intestine is that an injection in the lumen

of the intestines can be given directly In both these methods the animal was trained to lie down on the table quietly and this can be easily done by training for a fortnight or three weeks

Inject ions of berberine sulphate given to such animals either subcutaneously or through the intestines do not show a very marked alteration in the normal movements of the intestines Sometimes the tone of the muscle is slightly increased, but usually there is no change in the amplitude of contractions of the pendular movements

The movements of the small intestines were also studied in the cat *in situ* by means of a Jackson's Enterograph Injections of berberine sulphate produced a momentary increase in the tone of the intestinal musculature and stimulation of the peristaltic movements followed by inhibition (Graph II, *a*) This effect, however, only lasted for a short time and after 2 to 3 minutes the gut resumed its normal tone and the movements became normal This increase of tone and acceleration of the movements were absent when the alkaloid was administered after atropine had been given in sufficiently large doses to paralyse the vagal nerve endings The alkaloid when injected subcutaneously or intramuscularly would, therefore, appear to stimulate the vagal nerve endings

The rhythmic contractions of the spleen are increased in very much the same way as by pentavalent compounds of antimony and this fact account for the provocative properties attributed to the alkaloid in chronic malaria

Action on the cardiovascular system —Berberine sulphate injected intravenously in animals under urethane or chlorolose anæsthesia produces a sharp fall of blood pressure (Graph II, *a* and *b*) The amount of fall and its duration depend upon the dose of the alkaloid With smaller doses such as 1 to 3 mg per kilo, the fall is sudden but the recovery is rapid With larger doses such as 5 to 10 mg per kilo the fall is marked and is maintained for a considerable time The fall is accompanied by an increase in the volumes of intra-abdominal organs, e g, the spleen, the kidney, the intestines, etc (Graph II, *a* and *c*), showing that the vessels of the splanchnic area are dilated

Perfusion of the blood vessels in frogs with 1 in 50,000 produces an increase in the flow of the perfusate showing that the vessels are dilated Perfusion experiments with the limb of a cat similarly show dilatation of blood vessels with dilutions higher than 1 in 10,000

The heart *in situ* is depressed by such doses of berberine sulphate as 3 to 5 mg per kilo In cardiometer experiments, slight dilatation of the heart is observed (Graph II, *b*), the myocardiograph experiments similarly show a depression of both the auricles and ventricles (Graph II, *f*)

Perfusion of isolated mammalian and amphibian hearts with such dilutions as 1 in 100,000 produces a definite depression (Graph II, *d* and *e*)

Respiratory system—According to Curci (1892) berberine sulphate in warm-blooded animals produces respiratory distress and later emphysema, oedema of the lungs occurs and the animal dies of respiratory failure

It has been already mentioned in connection with experiments dealing with the toxicity of the alkaloid, that one of the commonest findings in post-mortem examination after lethal doses is a marked congestion of the lungs often accompanied by hæmorrhages. Our findings, therefore, confirm the observations made by Curci

Small doses of berberine sulphate such as 1 to 2 mg per kilo produce an initial stimulation followed by a depression of respiration and subsequent recovery (Graph II, *a*). The initial stimulation may either be due to fall of blood-pressure or to the minute emboli formed in the lungs by precipitation of the alkaloid in the circulation in the same way as the organic compounds of arsenic and antimony. The depression following the initial stimulation may be of the nature of simple apnoea following hyperpnoea. With larger doses of berberine, however, the initial stimulation is followed by profound depression which ends in complete cessation of respiratory movements. This indicated that the alkaloid in larger doses may paralyse the respiratory centre, and we studied this effect in more detail by Thomas and Frank's method. The movements of the strip of diaphragm isolated by this method definitely show an initial stimulation (Graph I, *c*) followed after a time by marked depression. Introduction of very low concentrations of the alkaloid into the cisterna magna also produced a profound depression of respiratory movement followed by complete cessation. There appears to be no doubt that with toxic doses of this alkaloid death is due to respiratory failure and much larger doses are required to kill the animal if artificial respiration is maintained. All these experiments point to the fact that berberine in larger doses has a marked depressant action on the respiration. In smaller doses berberine sulphate produces a constriction of the bronchioles and spasmodic breathing (Graph II, *a*).

Genito-urinary system—In our experiments spontaneous evacuation of the bladder contents was noticed whenever a large dose of berberine sulphate was given intravenously, particularly if the bladder was previously in a distended condition. The movements of the bladder, when recorded *in situ*, showed an increase of tone and contractions (Graph I, *d*). The secretion of the urine is slightly diminished, but the effect appears to be mainly vascular for as soon as the blood pressure regains its normal level the rate of secretion becomes normal.

The movements of the uterus, *in situ*, are increased with 2 to 4 mg per kilo body weight (Graph I, *e*). In perfusion experiments addition of berberine sulphate in concentrations of 1 in 100,000 or more produced an increase of tone and stimulated the rhythmic contraction of the uterus (Graph I, *f*).

Excretion—After absorption little or no alkaloid can be detected in the urine in experimental animals. Post-mortem examination of the kidneys of experimental

animals also lends support to the view that the drug is probably not excreted through the kidneys. No evidence could be obtained of the drug being excreted in the gastro-intestinal tract when given by the subcutaneous or the intramuscular route. In man, however, the alkaloid can be detected in the urine in appreciable quantities after administration by the mouth. A portion of the alkaloid is also oxidized in the body as was originally suggested by Berg.

Discussion—A definite and sharp fall of blood-pressure is observed in experimental animals after doses varying from 0.0015 to 0.002 g per kilo of berberine sulphate. This fall is associated with an increase in the volume of the spleen, and the intestines and to a lesser extent in the kidney volume. The fall in blood pressure is, therefore, associated with dilatation of the vessels of the splanchnic area associated with depression of the contractions of the auricles and the ventricles and a dilatation of the heart. The fall in blood-pressure is less marked or may be entirely absent in pithed animals and is probably central in origin. As there is a small rise in the volumes, the spleen and the intestines even after paralysing doses of ergotoxin, the drug may also produce vasodilatation through direct depression of the muscles of the vessel wall. This possibility is strengthened by the fact that berberine has a definite dilating effect on the blood vessels of the isolated limb. Perfusion experiments also confirm the depressant action of the alkaloid on the vasomotor centre.

On the smooth muscles of the intestine, bladder, uterus, etc., the alkaloid acts probably mainly through the parasympathetic endings as this action was abolished by atropine sulphate.

Therapeutic uses of berberine—Berberine containing plants have been used by both the Hindu and Mohammedan physicians as a stomachic and bitter tonic in the same way as quassia and calumba. They have also been used as antiperiodic, alterative in remittent types of fevers. The root paste is used as a paint for acute ophthalmia in children. It has also been used in the treatment of leprosy, snake bite, jaundice and vomiting of pregnancy. The fruit or berries of *B. asiatica* are given as a cooling laxative in children, the stems are said to be diaphoretic and laxative and are recommended in rheumatism. The root bark is rich in bitter principles and is used as a tonic and antiperiodic. The crude extract, *rasaut*, is given as a purgative in children as a blood purifier and as an external application in conjunctivitis in combination with opium. As a local application it is used as an application for indolent ulcers and it has also been recommended for gastric and duodenal ulcers.

Berberine has been used by physicians practising the Western system against fever of intermittent types, malaria, vomiting of pregnancy and diarrhoea in doses of 2 to 6 grains in cachets. Sabestane (1926) used berberine as a provocative for the diagnosis of latent malaria. Percy Andre (1927) advocated the hydrochloride in cases of malarial splenomegaly. Jolly (1917) first tried *rasaut* in the treatment

of oriental sore and since then other workers have confirmed its utility in this condition

Malaria—Berberine and its compounds have been reputed to have effective antiperiodic properties in the indigenous medicine. The Indian physicians used berberine salts in cachets in the treatment of malaria in doses of 2 to 6 grains. The sulphate in doses of 3 to 5 grains three times a day was tried by the senior author in the treatment of a series of cases of malaria in the Carmichael Hospital for Tropical Diseases. Doses of 3 grains three times a day on 3 consecutive days failed to arrest paroxysms of malarial fevers and microscopic examination of the blood revealed no change in the number of malarial parasites present. In 9 cases in which it was tried, in no instance was there any change in the signs and symptoms produced by the diseases. All infections whether those with *P. malaria*, *P. vivax* or *P. falciparum* remained unaffected by this alkaloid. Quinine administration in adequate doses in these very cases controlled the temperature and caused the disappearance of the parasites from the blood.

Dermal leishmaniasis—Das Gupta and Dikshit (1929) gave local injections of berberine sulphate in the treatment of oriental sore and found it superior to injections of antimony compounds. A 2 per cent solution of the sulphate was used for infiltrating the margin of the ulcer, the treatment being repeated every week. As a rule not more than 2 to 3 injections were required to cure the sores which had resisted treatment with carbon dioxide snow, local applications of tartar emetic ointment and intravenous injections of antimonials and other measures. The advantage of this drug lies in the fact that it produces a rapid and painless cure of oriental sore and the cost of treatment is very small.

SUMMARY AND CONCLUSIONS

(1) Berberine is not a very toxic alkaloid. The M L D for frogs and rats being 0.1 mg and 0.25 mg per gramme of body weight, the M L D for rabbits when given subcutaneously is 0.1 mg per gramme of body weight.

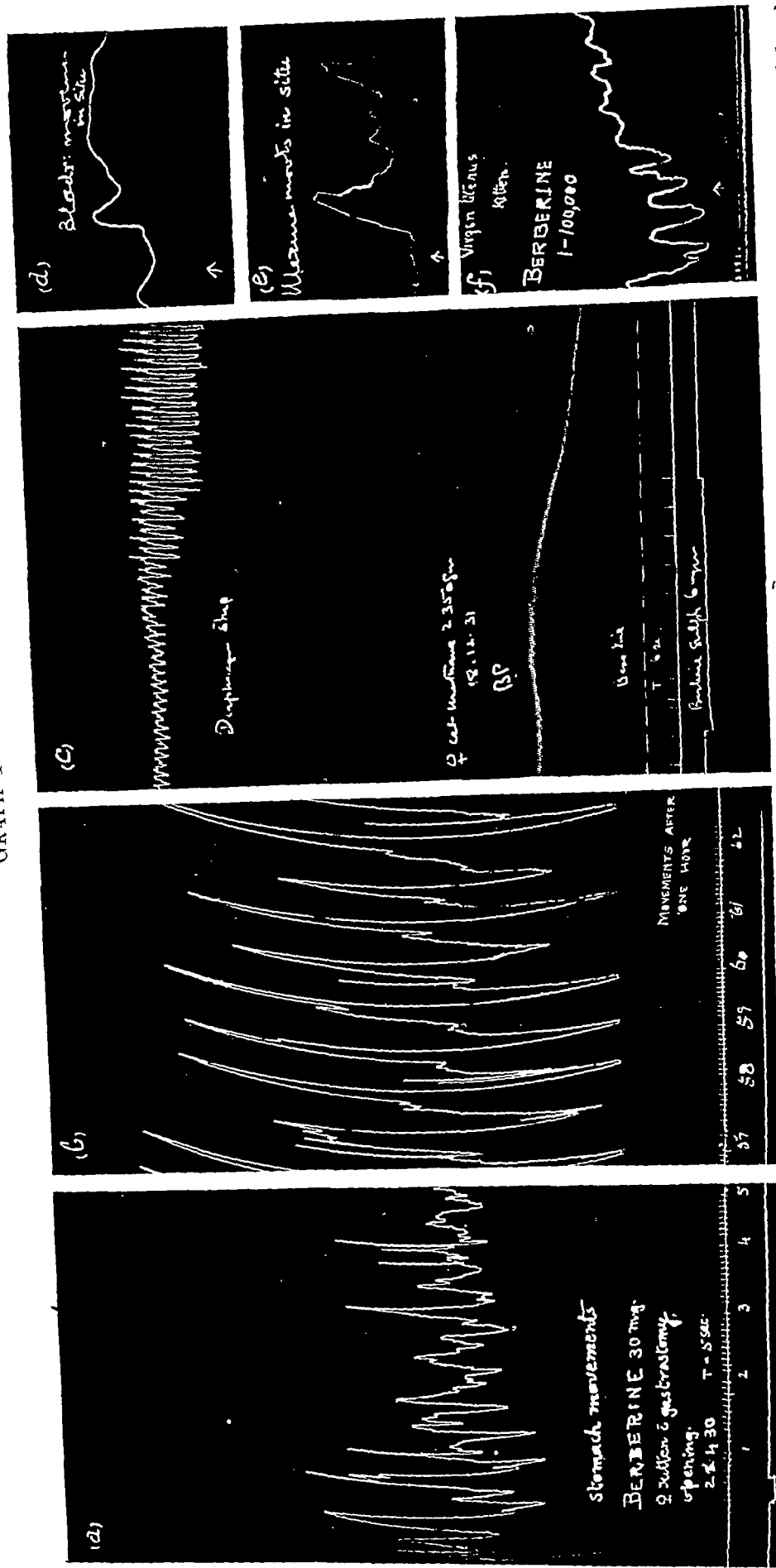
(2) The drug is readily absorbed from the gastro-intestinal tract and by the subcutaneous and intramuscular routes.

(3) It appears to be completely broken up in the body in rabbits as it can neither be detected in the faeces, nor in the urine and other excretions. In man however, the alkaloid is found in the urine within a few hours after administration by the mouth.

(4) It has little or no effect on the automatic movements of the gastro-intestinal tract when introduced into the lumen of the gut. Intravenous injections produce a momentary increase in the tone and peristaltic movements.

(5) Berberine has a depressant effect on the cardio vascular system and produces a sharp and persistent fall of blood-pressure. In 1 in 50,000 solution it depresses the amplitude and force of beats of the isolated mammalian heart,

GRAPH I



- Figs (a) and (b) show the movements of the stomach of a cat with a balloon inserted through a fistula. Note the normal movements become inhibited immediately after 30 mg of berberine sulphate is introduced into the stomach. There is a marked increase about an hour after when the alkaloid is absorbed and is circulating in the blood.
- Fig (c) shows the movements of the isolated strip of diaphragm with phrenic nerve intact (Thomas and Frank's method) and blood-pressure in a cat. Injection of 6 mg of berberine sulphate produces stimulation of the diaphragmatic movements and a fall of blood-pressure.
- Figs (d) and (e) show the increase in the tone and rhythmic contractions of bladder and uterus in a cat *in situ* after intravenous injections of 2 to 3 mg of berberine per kilo.
- Fig (f) shows the movements of the perfused virgin uterus of a kitten. Note a marked increase in tone.

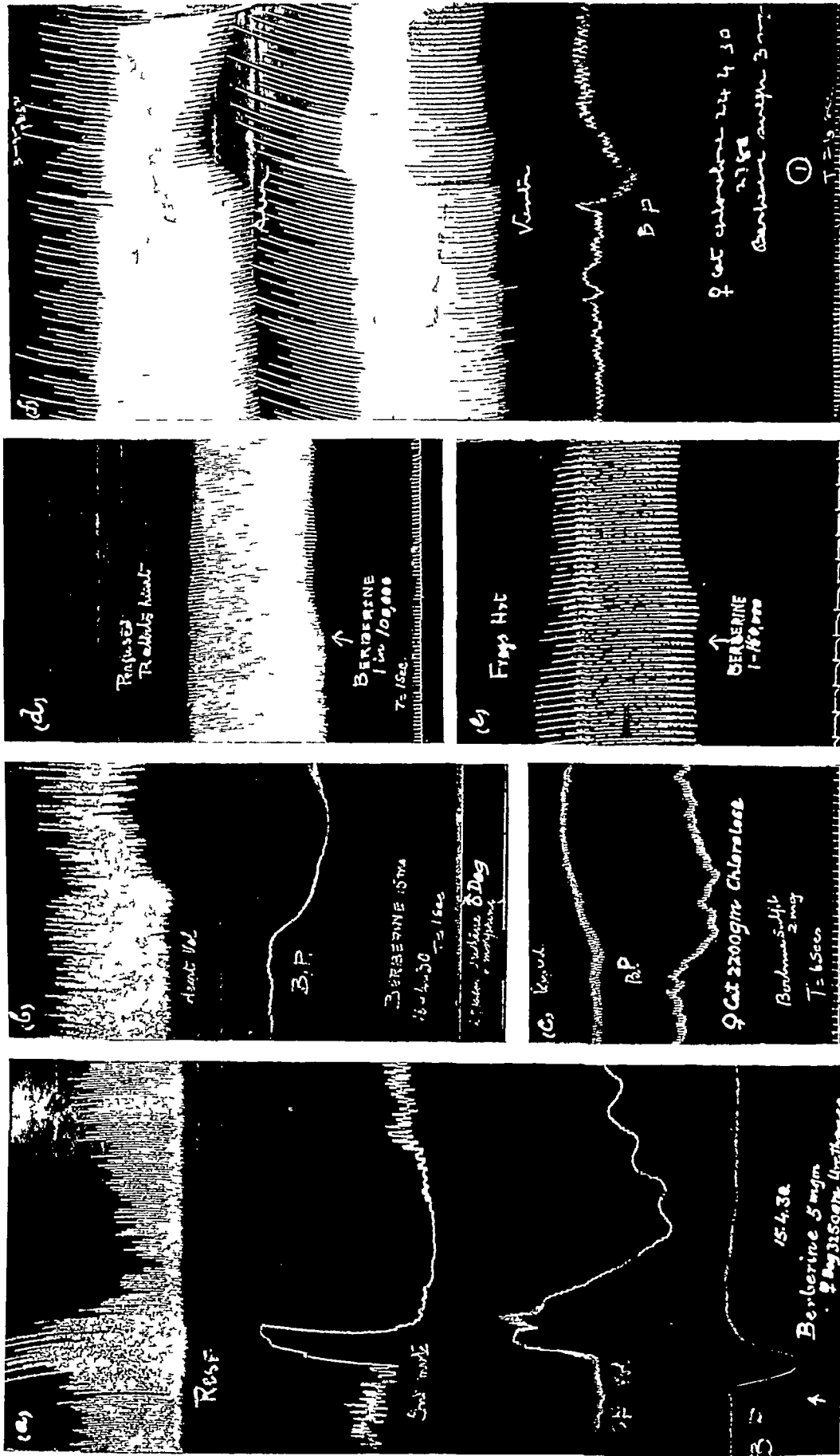


Fig (a) From above downwards, respiration, intestinal movements, spleen volume and blood pressure in a dog. Note that the respiration is at first stimulated, then depressed; the intestines show a momentary contraction followed by inhibition of movements, the spleen volume shows marked dilatation and increase of rhythmic movements.

Fig (b) shows heart volume recorded by a cardiometer and blood pressure in a dog. 15 mg of berberine produced depression of the heart and dilatation of the organ; the B.P. falls.

Fig (c) shows kidney volume and blood pressure in a female cat. 2 mg of berberine sulphate produce an increase in the kidney volume and a fall of blood pressure.

Fig (d) shows the depression of the isolated rabbit's heart after perfusion with 1 in 100,000 berberine sulphate.

Fig (e) shows depression of a frog's heart after perfusion with 1 in 150,000 berberine sulphate.

Fig (f) myocardiograph tracings. From above downwards: auricular contractions, ventricular contractions and blood-pressure. Injection of 3 mg of berberine sulphate shows depression of both the auricles and the ventricles.

myocardiograph experiments show slight depression of both auricles and ventricles, cardiometer experiments show a definite dilatation of the heart

(6) The vasomotor centre is depressed The blood vessels of the body generally and of the splanchnic area particularly are dilated

(7) The respiratory system is very susceptible to the drug, small doses stimulate respiratory centre producing acceleration of the movements, with larger doses there is initial stimulation followed by depression Very large doses produce death by paralysing the respiration, the heart goes on beating long after the respiration stops

(8) The contractions of the uterus and the bladder are stimulated

(9) Berberine has been clinically tried in a series of cases of malaria in doses ranging from 3 to 5 grains, three times a day. It has no effect whatsoever on the clinical signs and symptoms or parasites of any forms of malaria

(10) The alkaloid has been shown to have a remarkable effect on *Leishmania tropica* which produces oriental sore. In such dilutions as 1 in 80 000 of the alkaloid the growth of leishmania in modified N N N media is definitely inhibited. A 2 per cent solution injected at the base of the sore produces healing after 2 or 3 injections

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A NOTE ON THE BLOOD CHOLESTEROL IN OSTEOMALACIA

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[Received for publication, January 26, 1932]

In the course of previous work on osteomalacia (Hughes and others, 1929 and 1930) it was found that the blood cholesterol was below normal in some cases of this disease. The cholesterol was estimated by the method of Leiboff (1924) in which only a small quantity of blood or plasma (0.25 c.c.) is extracted and the extraction is carried out without previous drying. By this method figures varying from 114 to 160 mg per 100 c.c. of whole blood were obtained in ten normal persons. As, however, the cholesterol content of the corpuscles is practically constant in health and disease, determinations are now usually carried out in plasma only and Gardner and Gainsborough (1927) using the very accurate digitonin method found the limits of normality for plasma to be 0.104 and 0.223 g per cent in the case of males and 0.078 and 0.227 g per cent in the case of females.

With a view to assessing the value of our earlier results we have recently made further observations on the plasma cholesterol in osteomalacia using both Leiboff's method and the plaster of paris method of Myers and Wardell (1918). Our patients would not allow sufficient blood to be taken to permit of the digitonin method being employed and the free and ester cholesterol were therefore not separately estimated. Hence our figures are still of limited value. Still, as far as they go, they confirm the occurrence of low total plasma cholesterol in certain cases of osteomalacia. They further indicate that the method of Leiboff, when carefully carried out according to the original description and with due regard to the factors affecting the accuracy of the colorimetric estimation of cholesterol, as enunciated by Gardner and Williams (1921), gives results

(1205)

which correspond fairly closely with those obtained by the plaster of paris method. Our previous figures can therefore be regarded as at least a tolerably close approximation of the actual amount of cholesterol in the samples of blood or plasma examined. Gardner and Williams (1921) have shown that in the colorimetric estimation of cholesterol it is essential to work at a constant temperature, that the amount of H_2SO_4 determines the rapidity of development of the blue colour and to some extent its quality and that it is advisable to use concentrated H_2SO_4 so as to exclude traces of water. The amount of acetic anhydride added does not affect the result as long as there is an excess. The following are comparative values obtained with Leiboff's and Myers and Wardell's method —

Leiboff (mg per 100 c c)	Myers and Wardell (mg per 100 c c)
61	81
171	142
114	135
122	112
214	202
182	143
205	202
182	143
117	111
192	169

It is seen that Leiboff's technique gives a higher result than Wardell's in the majority of cases.

Among 15 cases of osteomalacia in whom the cholesterol content of the whole blood was determined by Leiboff's method only (Hughes and others, 1929) there were 10 in whom the value was less than 100 mg and 3 in whom it was less than 80 mg per 100 c c. Plasma determinations on 12 others by both methods showed a value below 100 mg in 5 cases in all of whom except one it was below 80 mg. Out of 27 cases, therefore, there was a definitely subnormal level in 7 and a very low normal level in 8. Although a low value was found only in severe cases some patients with normal or even super-normal plasma cholesterol also showed marked symptoms and signs.

The significance of the low blood cholesterol is not at all clear. It is perhaps natural to assume that it is in some way associated with the vitamin (A and D) deficiency that exists in this disease. Vitamin D has been shown by Harris and Innes (1931) not only to cause calcification of cartilage but to increase the absorption of Ca (or P) from the intestine or to diminish excretion into the gut. It is possible that it is also concerned with the absorption and retention of cholesterol, especially of the ester fraction. Gardner and Gainsborough (1930) have demonstrated a low plasma content of ester cholesterol in the early stages of biliary obstruction and

in complete biliary fistula and they attribute this to defective absorption from the intestine. The free cholesterol in these conditions is normal or slightly increased*.

A low plasma cholesterol occurs in conditions of pronounced cachexia and lowered vitality in marked anæmia and in febrile infectious diseases but we found the general state of nutrition and the condition of the blood to be no worse in the patients with hypo-cholesterolæmia than in others whose cholesterol was normal or above normal. Nor did any patient suffer from pyrexia with the exception of one who from time to time had bouts of low fever (99 F or so) of obscure origin.

TABLE

Effect of treatment on the blood chemistry in 3 low cholesterol cases

Number	Date	Age	Age at onset	Clinical notes	Calcium in mg per 100 c.c. of serum	Inorganic phosphorus in mg per 100 c.c. of serum	Cholesterol in mg per 100 c.c. of plasma
1	14-11-30	25	19	Developed pains in the feet six years ago. Weakness in legs and backache occurred during first pregnancy 3 years ago. Became worse after delivery. Diet poor—contained hardly any milk and little ghee.	11.4	2.75	39
	29-11-30				11.6	2.40	94
	17-12-30				10.8	3.30	42
	3-1-31				11.6	3.30	72
	14-1-31				10.8	3.90	43
	3-2-31				11.7	5.40	114
2	16-12-30	18	15	Symptoms began with severe backache 3 years ago during pregnancy, became worse after premature delivery. Legs contracted. Diet poor—contained little milk or milk products.	9.1	3.10	61
	5-1-31				10.0	3.90	69
	15-1-31				9.6	4.60	71
	10-2-31				9.8	5.50	75
	11-3-31				10.3	5.40	95
3	31-1-31	22	21	Backache and pains in the limbs began after childbirth. Consumes only small quantities of milk.	9.8	3.57	42
	17-2-31				9.8	6.16	60
	12-3-31				10.3	5.74	92

* McGowan and others (1931) have adduced evidence to show that the probable mode of action of vitamin D in the cure and prevention of rickets, the pathogenesis of which is similar to that of osteomalacia, is to set free from the lipins of the body inorganic phosphorus the relative deficiency of which they consider to be the essential cause of rickets. Of interest in this connection is the fact that we found (Hughes and others, 1931) the best response to treatment in osteomalacia to be associated with a normal serum calcium and a serum inorganic phosphorus as high as that found in infants and young children.

The effect of treatment with vitamins A and D and calcium glycerophosphate is illustrated in the table (3 cases). Improvement in the clinical condition was associated with a rise in the cholesterol which was roughly parallel to the rise in inorganic phosphorus, although fluctuations sometimes occurred.

SUMMARY

The occurrence of low plasma cholesterol in some cases of osteomalacia has been confirmed.

Our thanks are due to Major H. S. Anand, I.M.S., Professor of Physiology, for permission to work in his Laboratory.

The expenses of this research were defrayed by the Indian Research Fund Association.

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STUDIES IN THE PARASITOLOGY OF MALARIA IN THE FEDERATED MALAY STATES BETWEEN 1900-1912

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[Received for publication, January 28, 1932]

LIEUT -COLONEL KNOWLES, I M S , and Mr R Senior-White, F R S E , have placed all workers on malaria under a deep debt of gratitude for the 'Studies in the Parasitology of Malaria' published as *Memor* No 18 of the *Indrian Medical Research Memours*, December 1930. At what must have been enormous labour, they have given those interested in malaria a mass of information which they could never have obtained for themselves. Hundreds of medical journals were searched, but much of the information was buried in Government reports, some of which were available only to local workers. They say 'And when we came to study the parasitology of world malaria, we gradually realized the amazing state of affairs in the literature. Throughout the tropics, wherever there is a French or Dutch Colony, malaria has usually been extensively investigated in that Colony, and the results published in well-known and readily accessible journals. But for the British Empire the information is scanty in the extreme'. The authors call for information to fill the lacunæ in our geographical knowledge, and write (p 399), 'Work in the Federated Malay States appears to have ignored the parasitological aspects of malaria, almost completely'.

For the sake of the British Empire I am glad to say that in Malaya, in addition to its unique and happy record of combined scientific and practical anti-malarial work, a good deal of research has been done on the malaria parasites. But it was begun so long ago as to be almost forgotten history, and as in India, much of it was not published in readily accessible journals, and some is buried in official reports.

Probably the first reference to malaria parasites in the F M S is in Vol. I, No 1, of 'Studies from the Institute for Medical Research', Kuala Lumpur, Federated

Malay States, by Dr Hamilton Wright(1), Director It is dated August 1901
The title of the volume is 'The Malarial Fevers of British Malaya' He says —

TABLE I

' Out of 251 cases of fever personally studied by me —

93 were Malignant Tertian

78 „ Benign Tertian

56 „ Quartan

22 „ Pigmented Quotidian

2 „ Unpigmented Quotidian '

To this volume I(2) contributed a note on parasites and their percentages
The latter are included in the next reference—which is the Selangor Medical
Report(3) for the year 1901 Selangor is one of the states of the four Federated
Malay States In it the State Surgeon, Dr E A O Travers, wrote —

' Since 1st July all cases of malarial fever admitted to those hospitals in
charge of District Surgeons have been diagnosed by the microscope '

' The figures for the General Hospital, Kuala Lumpur, and the District
Hospital, Klang, are as follows —

TABLE II

	Total	District Hospital, Klang	General Hospital, Kuala Lumpur
Malignant	188	131	57
Deaths	27	26	1
Percentage of deaths	14.3	19.8	1.8
Benign Tertian	80	48	32
Deaths	2	2	0
Percentage of deaths	2.5	4.1	0
Benign Quartan	12	5	7
Deaths	0	0	0
Percentage of deaths	0	0	0

' As most of the hospitals are under the care of untrained men, it is impossible to carry out the diagnosis of all cases of malarial fever by the microscope '

' The above figures, however, show clearly that all varieties of malarial fever are met with in Selangor, also that, as in other parts of the world, the malignant type is the more common and the most fatal, whereas the quartan type is the rarest and the least dangerous '

In 1901 the microscopic examinations at Klang were made by myself, and those at Kuala Lumpur by Dr Travers(4) Both of us had been at the London School of Tropical Medicine and knew our parasites

In the following year, 1902, the figures for the whole State were as follows —

TABLE III

Malignant	240
Tertian	181
Quartan	86
Mixed Infection	13
Type undiagnosed	2,077

They include those for my hospital at Klang During 1901 and 1902 the results of my own personal examinations alone were recorded As a busy District Surgeon in charge of a considerable district and often away from headquarters, it was of course impossible for me to examine every malarial blood personally A certain amount of further information is given in a note in my own hand-writing entitled ' Classification of Malaria at Klang Hospital, 1901 and 1902 ' The note which is before me as I write, fortunately survived a white-ant disaster when I was on leave in 1908 It shows among other things the monthly distribution of the parasites The note also states that the blood examinations ceased on 4th November, 1901, and were not resumed until the end of February 1902 (It was at the end of 1901 that the outbreak of malaria almost closed Port Swettenham)

The Table IV shows the number of cases diagnosed as malaria but also where the type was not determined

During 1901 and 1902, I trained my assistant, Mr R W B Lazaroo He learned quickly, and as I found him thoroughly reliable in this, as in other matters, the examination of malarial blood in Klang Hospital was in his hands from 1903 onwards About 1909 he was transferred to Kuala Lumpur where he afterwards worked under Dr William Fletcher, with such merit that on his retirement he was awarded the Imperial Service Medal If the monthly returns of malaria from the District Hospital, Klang, from 1903 to 1907 have been spared by white ants and other destructive agencies in the Stores of the Medical Department of Selangor at Kuala Lumpur there is contained in them an early record of malaria in Klang, which it might be of interest to recover, and for the accuracy of which I can vouch

KLANG HOSPITAL, 1902

TABLE IV

Malaria

	M	M and B T	M and B Q	B Q	B T	B T and B Q	Total type diag nosed	Type undiag nosed	Grand Total	REMARKS
January								74	74	
February	4			1	5		10	35	45	Examination began at end of month
March	5	1		6	5		17	17	36	On leaving spleen examination stained
April	7	1		7	2		17	9	26	
May	13			6	3		22	10	32	
June	5a			4b	3		12	12	24	(a) 1 with beri beri (b) 1 with nephritis
July	12		1	4	6a		23	8	31	(a) 1 with nephritis
August	11			1	3		15	14	29	
September	10			2a	10		22	14	36	(a) 1 with diarrhoea
October	9	1			3		13	15a	28	(a) 1 died within $\frac{1}{2}$ hour
November	3a	1		3	2	1	10	15	25	(a) 1 with diarrhoea
December	7			2	2		11	16a	17	(a) 1 case coma
	86	4	1	36	44	1	172	241	403	

Early in 1903 Dr William Fletcher(5) arrived, and the number able to identify malarial parasites and train subordinates to do so was augmented. I have no copy of the Selangor Medical Report for 1903, but in the report for 1905 Dr William Fletcher gives the types treated at the General Hospital, Kuala Lumpur, as Table V —

TABLE V

Type	1904	1905
Malignant Tertian	137	191
Benign Tertian and Mixed	42	40
Quartan	2	7
Type undiagnosed	124	70

In the report for 1905 the figures for the District Hospital under Dr McClosky(6) are given in Table VI —

TABLE VI

Type	1905	1904	1903	1902
Malignant	279	193	136	81
Quartan	53	55	79	55
Benign Tertian	56	35	26	43
Mixed types	9		12	

The mixed types were —

Malignant and Benign Tertian	7
Malignant and Quartan	2

' It will be observed that the order of prevalence of the Quartan and Benign Tertian is reversed this year '

From Volume III of *Studies from the Institute for Medical Research, 1908* ' by Dr Daniels(8), I extract the following —

' Malarial Parasites are widely but not uniformly distributed The distribution of the different species as seen in the District Hospital in Kuala Lumpur are shown by Dr McClosky to be as follows —

Malaria at the District Hospital, Kuala Lumpur, during 1903
by A J McClosky(7), M B, C M (Edin)

1 Microscopical examination of the blood of all cases of malaria was made at the Institute for Medical Research, and a diagnosis made according to the parasites found

2 Two hundred and fifty-three cases were treated during the year, classified according to the following types Malignant, Quartan, Benign Tertian and Mixed types

The following, Table VII, shows the number and percentage of each type and compared with the preceding year —

TABLE VII

Types	1903		1902	
	Number	Percentage	Number	Percentage
Malignant	136	53.73	77	43.57
Quartan	79	31.22	55	30.72
Benign Tertian	26	10.27	41	22.9
Malignant and Benign Tertian	10	3.95	3	1.14
„ and Quartan	1	.39	1	.55
Benign Tertian and Quartan	1	.39	2	1.11
TOTAL	253		179	

There has been a marked increase of malaria this year as compared with the previous year. This increase is mainly due to the large number of admissions from amongst the coolies working at Ulu Gombak. The order of prevalence of the different types of fever is the same for both years, and does not correspond with that given by Dr Hamilton Wright in his Study No. 1 in which the Benign Tertian is shown to be more prevalent than the Quartan type.

The districts from which most of the cases came are as follows (Table VIII) —

TABLE VIII

		Quartan	Benign Tertian	Malignant Tertian	PER CENT		
					4	3	1
					Quartan	Tertian	Malignant Tertian
Kuala Lumpur	66	20	13	33	30.3	19.0	50
Gombak	45	12	3	30	26.6	6.6	66.6
Ampang	28	9	1	18	32	3	64
Kepong	12	5	1	6			
Panthal	10	1	2	7			
Pudoh	8	2		6			
Ulu Klang	7	1	1	5			
Sungei Bost	7	3		4			
Simpang	7	1	2	4			
Setapah	6	1		5			
Kajang	9	6	1	2			
TOTAL					29.7	11.7	58.5

The particular streets in Kuala Lumpur from which the patients came could not be ascertained in all cases, but those obtainable were as follows —

Batu Road 18, Java Street 11, Brickfields 8, High Street 3

The following, Table IX, shows the monthly admissions and rainfall —

TABLE IX

Months	1903		1902	
	Admissions	Rainfall	Admissions	Rainfall
January	17	4 99	17	2 76
February	15	3 68	6	9 43
March	19	9 19	7	6 91
April	28	7 03	13	13 71
May	20	13 81	10	9 99
June	25	4 40	12	6 99
July	21	1 97	14	3 19
August	12	6 70	17	7 46
September	22	4 51	22	5 48
October	18	11 58	17	18 18
November	19	8 82	11	19 48
December	25	12 55	10	11 80
TOTAL	241	89 23	166	115 38

The only deduction which can be drawn from the above table is that in each year the month in which the rainfall was lowest, viz, July and January respectively, was followed by the month in which the admission rate was lowest

The following, Table X, shows that in the past two years there has been no great seasonal variation in the prevalence of the parasites —

TABLE X

Months	Malignant	Quartan	Benign Tertian	Malignant and Benign Tertian	Malignant and Quartan	Quartan and Benign Tertian	TOTAL	Malignant	Quartan	Benign Tertian	Malignant and Benign Tertian	Malignant and Quartan	Quartan and Benign Tertian	TOTAL
January	6	7	4				17	4	8	5				17
February	5	6	4				15	8	4	4				16
March	8	8	3				19	3	1	2			1	7
April	11	11	2	3			28	7	4	3				14
May	7	7	1	3		1	20	3	4	2			1	10
June	14	10	1				25	7	2	2	1			12
July	15	3	1	1	1		21	2		12				14
August	10	1	1				12	12	4	1				17
September	14	5	1	2			22	15	5		1	1		22
October	15	2	1				18	8	7	2				17
November	12	6	1				19	2	3	5	1			11
December	18	4	2	1			25	1	9					10

Admissions of the Malignant type were more numerous during the last six months of the year, as the following figures show —

	1903	1902
1st six months	51	33
2nd „ „	84	43

' Malignant Parasite — This is the most common parasite met with at the District Hospital. Doubly infected corpuscles were frequently met with, and I have seen as many as five parasites in one corpuscle. Examinations of fresh blood films showed (1) young active amœboid forms, (2) quiescent ringlets with and without pigment, pigment generally active, as fine granules or rods, (3) brassy bodies, (4) crescents and globular bodies of the crescent series.

' Quartan Parasite — Single infections are more common than in the Malignant and Benign Tertian fevers. Fifty-three per cent of the cases were single infections.

Out of 41 cases, 22 were single infections, 11 double and 8 triple. Examination of fresh blood films showed (1) active young amœboid forms (2) pigmented parasites, pigment sluggish and in form of fine rods, (3) corpuscles not enlarged and not changed in colour, (4) sporulating bodies.

‘*Benign Tertian Parasites*—Double infection is common. Out of 21 cases, 14 were double and 7 single infections.

‘Examinations of fresh blood films showed (1) young active amœboid forms (2) pigmented parasites, pigment very active, and in form of fine granules, (3) hosts enlarged and pale, (4) sporulating bodies, (5) extra corpuscular forms, (6) small free pigmented bodies with the pigment very active.

‘It may be noted that the prevalence of Quartan shown in Dr McClosky’s report is not universal throughout the Federated Malay States. It is rare in Kuala Lumpur amongst Europeans and amongst the better class natives who are admitted to the General Hospital. Out of 255 cases in which the blood was examined the proportions were Quartan 2 per cent, Tertian 40 per cent, and Malignant Tertian 58 per cent. It is rare in other districts such as Klang.

‘Dr H. Wright (*The Malarial Fevers of British Malaya, Studies from the Institute for Medical Research, No. 1, Vol. 1*) gives out of 251 cases percentages of 22.3 per cent Quartan, 31 per cent Benign Tertian and 46.2 per cent of other types.

‘Of the cases of malaria treated at the District Hospital, only 28.8 per cent were from Kuala Lumpur. Of the remainder 45, or 23 per cent of the total, were from Gombak, a district where extensive works in partially cleared jungle are being carried out. Under such circumstances in Malaya, malaria is as severe and prevalent as in bad parts of Africa.’

In other States of the Federation, Dr Braddon(9) and Dr J. Tertius Clarke(10) were using the microscope as a routine, and training subordinates to use it. Indeed in the first decade of the century men in the Federated Malay States were using the microscope to a greater degree than I found in some other countries twenty years later. Some results were published but more, as I said, remained in unpublished reports, as the novelty of detecting malarial parasites passed off, and the practice became a routine in all the larger hospitals. Many of these figures should still be available and might be obtained, if application were made to the Senior Medical Officers of the States of Perak, Selangor or Negri Sembilan.

SINGAPORE

In addition to the figures in the *Memorandum* given by Dr Hunter for Singapore the following are from a printed ‘Report on Malaria’, 1911 by Dr Middleton(11), Municipal Health Officer, Singapore, one of Dr Hunter’s predecessors. The Report, which is very comprehensive, was prepared at the time that I was

asked to draw up an anti-malarial scheme for Singapore In that report Dr Middleton writes —

‘ In 1905 Dr Finlayson(12) found that out of 107 examinations “ practically all the cases showed Subtertian (or Malignant Tertian) infection ” In 4 years (1907 to 1910) the following results were obtained—out of 6,708 specimens examined, parasites were found in 4,430 cases and were classified as follows (Table XI) —

TABLE XI

Subtertian	3,341, or 75.4 per cent
Simple Tertian	707 „ 15.9 „
Simple Quartan	240 „ 5.4 „
Mixed Infection	140 „ 3.2 „
	<hr/>
	4,430 99.9
	<hr/>

‘ For the different years the proportions were as follows (Table XII) —

TABLE XII

	1907 Per cent	1908 Per cent	1910 Per cent
Subtertian	69.9	73.7	79.6
Simple Tertian	23.4	20.3	8.6
Simple Quartan	5.1	5.3	5.2
Mixed Infection	1.4	0.4	5.8

‘ The year 1909 is omitted for comparison because the total number of examinations was too small to yield reliable results The mixed infections consisted of Simple and Subtertian forms It partly accounts for the decrease in pure Simple Tertian infections and shows that the increase in the Subtertian form is greater than appears from the percentage prevalence of this form alone ’

‘ It will be seen that while Quartan infections have remained stationary and Simple Tertian have decreased by 11.8 per cent, Mixed infections and Subtertian

have increased by 4·4 per cent and 7·5 per cent respectively. That is to say, Malarial fever has been assuming a severer type year by year.

Dr Finlayson who made these examinations was Government Pathologist. It is interesting to compare the results of two sets of examinations —

	Dr Finlayson, 1907—1910	Dr Hunter, 1915—1928
Subtertian	3,341, or 75·4 per cent	2,130, or 40 per cent
Simple Tertian	707 „ 15·9 „	2,990 „ 57·1 „
Simple Quartan	240 „ 5·4 „	113 „ 2·1 „
Mixed Infection	140 „ 3·2 „	54
	4,430	5,284

The alteration in the percentage of the two species of parasites is very striking.

One cannot help asking what connection, if any, is there between the alterations of percentages and the great reduction in the death-rate from malaria and its associated diseases which followed the anti-malarial work so ably begun by Dr Middleton at the end of 1911, and continued by Dr Hunter.

Chart 1 shows the average monthly death-rates from all causes for the City of Singapore from 1903—1912. There was no anti-malarial work, and the great *A. maculatus* wave during this period is well seen. The little rise in the month of October is probably the result of malaria due to *A. umbrosus*.

In the curve for the years 1913—1922 the rise in the month of October is due to the influenza epidemic of 1918.

The curve for the year 1929 shows that malaria is still less, and as Dr Hunter now points out, there is very little actually contracted in the town and the cases that occur are mostly imported.

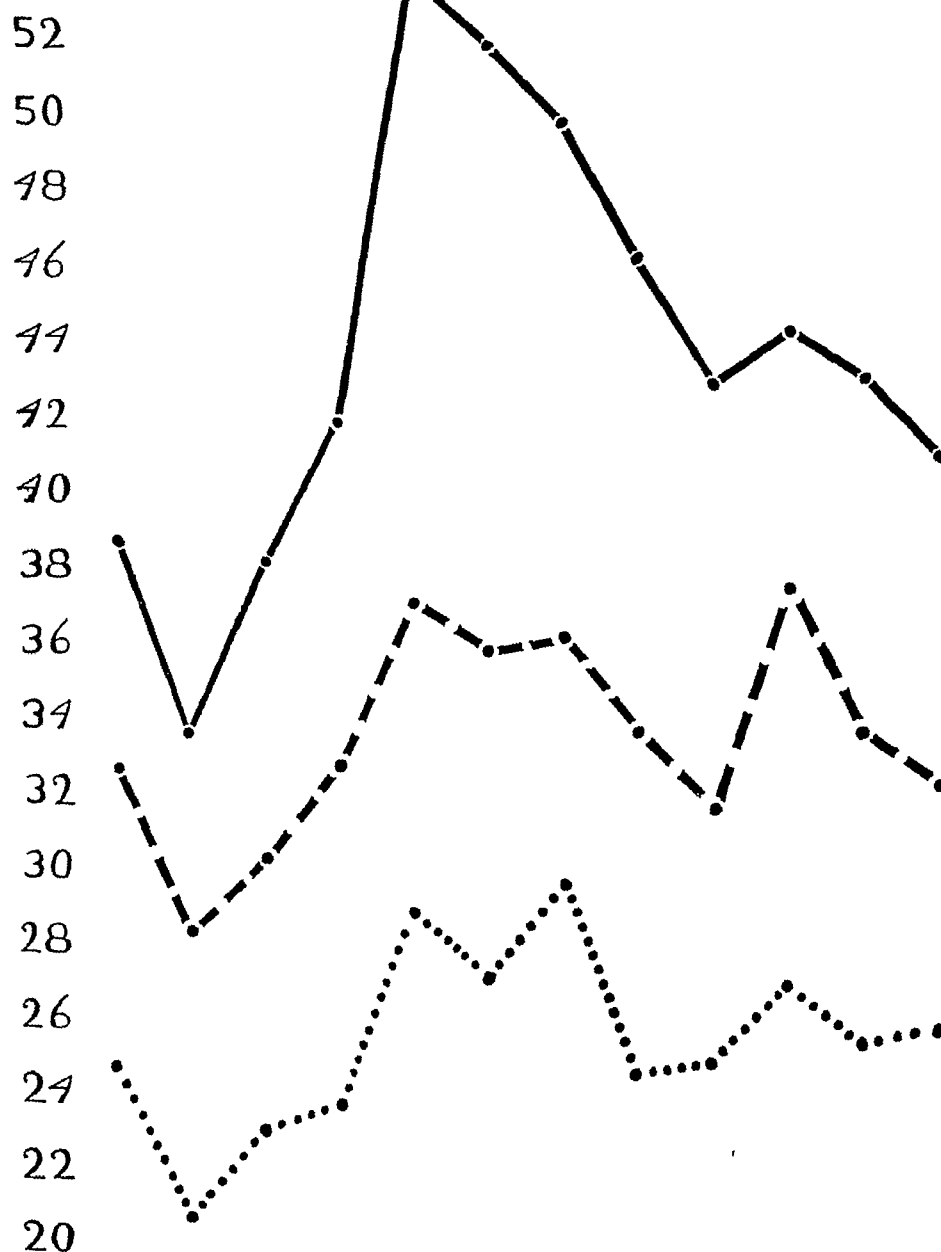
Malignant Malaria

In 1901 I discovered a case of malaria in which nearly 40 per cent of the blood corpuscles were infected. It was described in the *Journal of Tropical Medicine* for July 15th, 1903(13), under the title 'A Note on the Parasites of a Case of Malignant Malaria, with a Discussion on the Development of the Crescent.'

CHART 1

SINGAPORE AVERAGE MONTHLY DEATH RATE FROM ALL CAUSES.

54 JAN FEB MAR APR MAY JUN JUL AUG SEP. OCT NOV. DEC.



1903-1912. 1913-1922. 1929.

I note that 'where the film was thick hundreds of parasites could be seen in a single field. In many corpuscles a number of young parasites were seen, in one six were counted. Another striking feature was the number of the mature forms present, even rosettes were common. In many places groups of as many as thirty almost fully-developed parasites were counted, reminding one of what is occasionally seen in cerebral and other capillaries.'

But of chief interest was the fact that not only were the asexual parasites from the youngest to the most mature stage visible, but the whole development of the gametes was also visible. I need not describe the development here because it is well known now, but this observation is of interest because, at the time I wrote, the origin of the crescent was still a matter of dispute. Bignami and Bastianelli held the view that, 'After a variable number of paroxysms a certain number of corpuscles with central pigment, instead of advancing to sporulation, take the ovoid or spindle form and develop into the falciform body.'

Mannaberg on the other hand thought that the crescent was the result of the joining together of two parasites. I was able to point out that 'the crescent is derived from a young amoeboid parasite and is at an early stage to be differentiated from the asexual parasites by the scattered pigment, which is relatively, and in the great majority of parasites, absolutely motionless, and by the pale staining protoplasm, the characteristics of the full-grown crescent.'

This was, I believe, the first time that all the stages of the gametes were seen in the peripheral blood in such profusion that the observer could easily distinguish and describe the complete development of the sexual and asexual forms. What I described there has been repeatedly confirmed, and there is, of course, no controversy on the question now.

A slide of this case was one of the cherished possessions of the late Dr C W Daniels at the London School of Tropical Medicine where I saw it as late as 1908. Dr E A O Travers had another. The slide was also seen by Dr H E Durham and many others in Malaya. No other case like this was published until, I think, 1909, when Cropper published one from Jerusalem, and about 1913 when W M James published a case from Panama. All this is very ancient history now, but it shows that even at this early stage, the study of the parasites of malaria were not being overlooked in Malaya as the *Memor* supposes.

Quartan Malaria

In the *Malayan Medical Journal* of 1904(14) I published a paper entitled 'Some Clinical Features of Quartan Malaria'. It was re-published in April of the following year in the *Indian Medical Gazette*. I refer to it now because the results

of analysing a series of 83 cases was to show that Quartan fever is likely to be overlooked. The opening paragraph is as follows —

‘The most striking feature of the series of cases of Quartan malaria on which my observations have been made has been the absence, in the majority, of the characteristic Quartan pyrexia. The extraordinary periodicity of the pyrexia, which gave a name to the fever in ages long by, and which enabled the physicians of those days to differentiate it from the host of other fevers, was absent in no less than 60·6 per cent of my cases. But for the examination of the blood, the essential element in many of the cases would have been overlooked, and the treatment futile.’

Also I point out that some of the cases were admitted, not complaining of fever but of other symptoms. Œdema was very prominent. Diarrhœa and dysentery was another group. Sometimes the complaint was of abscess or abscesses frequently of large size and comparatively painless.

I drew particular attention in this paper to the fact that in some cases ‘the brunt of the disease appears to fall on the kidneys and chronic parenchymatous nephritis ensues’. In a paper with the title ‘Nephritis and Quartan Fever’ published in the *Journal of Tropical Medicine* of May 1st, 1912, Dr J Tertius Clarke(15), Health Officer Perak South, Federated Malay States, gives a list of 62 cases, and says, ‘Every medical man knows that, given the malaria parasite, he may find albuminuria, but he does not know that given the albuminuria without fever, he may find malarial parasites—in over 50 per cent of the cases and of this 50 per cent almost 100 per cent are Quartan.’

I have referred to my Quartan paper because an analysis of the temperature charts of 66 cases shows ‘that in 18 per cent of the cases there was no pyrexia and in another 18 per cent the pyrexia occurred at long intervals and was sometimes not complained of by the patients at all’. Now this means that Quartan malaria is very liable to be overlooked. It masquerades in the guise of many other diseases. This being so, one wonders whether the percentage of Quartan parasites returned from any country and published in the *Memor* is not far below what it should be.

My own feeling is that, when the parasitology of malaria is studied more completely Quartan malaria will be found to be much more widespread than is now realized. It may be, as the writers of the *Memor* suggest, that Quartan malaria is a dying species. On the other hand, it may be a species which has learned the advantage of prolonging its periodic sporulation, thus it reduces its destructive action on its host. It has in fact learned to live a quiet life, spread over many months, unlike its cousin the Malignant parasite which lives a short and merry life and whose motto might well be—‘Let us eat, drink and be merry for to-morrow we and our host will die.’

But whatever is discovered in the future, our successors in the years to come will always be indebted to Colonel Knowles and Mr Senior-White for giving them so extensive a record of the past. I hope that the authors themselves will continue their researches on the lines they have already worked on. I trust too that both they and others will follow up with an investigation of the lacunæ of our knowledge which their *Memor* shows to be only too common. From what I have indicated above there is probably a mass of information in old official reports in Malaya, and presumably in some other countries, which would well repay investigation. Even now the *Memor* is a milestone, and without milestones it is hard to measure progress on the highway of knowledge. But it is even more, it is a stimulus to further effort, and an invaluable guide to all who are engaged in research on the parasitology of malaria.

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